DOCTORAL THESIS

Biological Effect Indicators: Influence of Environmental Parameters and Contaminants on Amphipods

Natalja Kolesova

TALLINNA TEHNIKAÜLIKOOL
TALLINN UNIVERSITY OF TECHNOLOGY
TALLINN 2024

TALLINN UNIVERSITY OF TECHNOLOGY
DOCTORAL THESIS
40/2024

Biological Effect Indicators: Influence of Environmental Parameters and Contaminants on Amphipods

NATALJA KOLESOVA

TALTECH PRESS

TALLINN UNIVERSITY OF TECHNOLOGY
School of Science
Department of Marine Systems
This dissertation was accepted for the defence of the degree of Doctor of Philosophy in Earth Sciences on 19/06/2024.

Supervisor: Assistant Prof. Sirje Sildever
Department of Marine Systems
School of Science
Tallinn University of Technology
Tallinn, Estonia

Opponents: Dr. Zhanna Tairova
Department of Ecoscience
Faculty of Technical Sciences
Aarhus University,
Roskilde, Denmark

Associate Prof. Arvo Tuvikene
Institute of Agricultural and Environmental Sciences
Estonian University of Life Sciences
Tartu, Estonia

Defence of the thesis: 27/08/2024, Tallinn

Declaration:
Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Natalja Kolesova

----
signature

European Union
European Regional
Development Fund

Investing
in your future

ISSN 2585-6898 (publication)
ISBN 978-9916-80-184-0 (publication)
ISSN 2585-6901 (PDF)
ISBN 978-9916-80-185-7 (PDF)
DOI https://doi.org/10.23658/taltech.40/2024
Printed by Koopia Niini & Rauam

Kolesova, N. (2024). *Biological Effect Indicators: Influence of Environmental Parameters and Contaminants on Amphipods* [TalTech Press]. https://doi.org/10.23658/taltech.40/2024

TALLINNA TEHNIKAÜLIKOOL
DOKTORITÖÖ
40/2024

Bioloogilise mõju indikaatorid: keskkonnaparameetrite ja ohtlike ainete mõju kirpvähilistele

NATALJA KOLESOVA

TALTECH KIRJASTUS

Contents

List of publications

I Kuprijanov, I., Väli, G., Sharov, A., Berezina, N., Liblik, T., Lips, U., Kolesova, N., Maanio, J., Junttila, V., Lips, I., 2021. **Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland**. Mar. Pollut. Bull. 170. https://doi.org/10.1016/j.marpolbul.2021.112642

II Kolesova, N., Sildever, S., Strode, E., Berezina, N., Sundelin, B., Lips, I., Kuprijanov, I., Buschmann, F., Gorokhova, E. (2024). **Linking contaminant exposure to embryo aberrations in sediment-dwelling amphipods: a multi-basin field study in the Baltic Sea.** Ecol. Indic. 160. https://doi.org/10.1016/j.ecolind.2024.111837

III Strode, E., Barda, I., Suhareva, N., Kolesova, N., Turja, R., Lehtonen K. (2023). **Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod *Monoporeia affinis* from the Gulf of Riga (Baltic Sea)**. Water 15(2), 248. https://doi.org/10.3390/w15020248

Author’s contribution to the publications

Contribution to the papers in this thesis are:

I The author participated in the initial sampling, data analysis, manuscript writing, and editing.

II The author planned the study, obtained the funding, and was responsible for sampling, analysis of samples and data, and writing the manuscript.

III The author contributed to the planning of the study, data validation, writing, and editing of the manuscript.

Abbreviations

AChE	Acetylcholinesterase
AICc	Akaike Information Criterion corrected for small sample size
ANT	Anthracene
As	Arsenic
BoS	Bothnian Sea
BSAP	Baltic Sea Action Plan
BTs	Bityltins
CAT	Catalase
Cd	Cadmium
Cu	Copper
D8	Descriptor 8
dbRDA	distance-based Redundancy Analysis
DBAHA	Dibenz(a,h)anthracene
DBT	Dibutyltin
DNA	Deoxyribonucleic acid
DW	Dry weight
EBM	Effect-based method
EG HAZ	Expert Group on Hazardous Substances
GES	Good Environmental Status
GoF	Gulf of Finland
GoR	Gulf of Riga
GR	Glutathione reductase
GST	Glutathione S-transferase
HBCDD	Hexabromocyclododecane
HELCOM	Helsinki Commission
Hg	Mercury
HOLAS 3	Third HELCOM holistic assessment of the Baltic Sea, 2016-2021
IMO	International Maritime Organisation
MARPOL	The International Convention for the Prevention of Pollution from Ships
MBT	Monobutyltin
MSFD	Marine Strategy Framework Directive
NBP	Northern Baltic Proper
Ni	Nickel
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-p-dioxins and dibenzofurans
PFAS	Per- and polyfluoroalkyl substances

PFOS	Perfluorooctane sulphonate
PLI	Pollution Load Index
ReproIND	Reproductive disorders: malformed embryos of amphipods indicator
TBT	Tributyltin
TOC	Total Organic Carbon
WFD	Water Framework Directive
WGB	Western Gotland Basin
Zn	Zinc
%AbEmb	Proportion of aberrant embryos
%Fem>1	Proportion of females with more than one aberrant embryo in their brood pouch

1. Introduction

1.1 Hazardous substances in the marine environment

Marine environments, particularly coastal areas worldwide, are heavily influenced by chemical pollution from anthropogenic activities (Bashir et al., 2020). In turn, pollution affects individual organisms, biological communities, and the functioning of entire ecosystems (Sumpter, 2009; Mearns et al., 2013; Johnston et al., 2015; Rocha et al., 2016). Hazardous substances can be highly toxic to aquatic organisms and cause adverse effects even in low concentrations (Antizar-Ladislao, 2008; Sunday et al., 2012; Santhosh et al., 2024). The detrimental effects of hazardous substances may be magnified by the synergistic effects of different chemicals as well as by bioaccumulation in aquatic organisms (Knutzen, 1995; Marston et al., 2001).

Global surveys of multiple potentially hazardous substances have revealed their presence and spread in marine environments worldwide (Stemmler and Lammel, 2009; Wang et al., 2011; Staniszewska et al., 2013; Castiglioni et al., 2015; Langston et al., 2015). Some of the most well-studied groups of hazardous substances that are widely distributed in marine environments include heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dioxins, per- and polyfluoroalkyl substances (PFAS), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), butyltins (BTs), and pharmaceuticals (Chaudry and Zwolsman, 2008; Di Leonardo et al., 2009; Fattore et al., 2018; Wang et al., 2019; Yamazaki et al., 2019; Zandaryaa and Frank-Kamenetsky, 2021; Yu et al., 2023). Although some hazardous substances have already been banned, such as persistent organic pollutants, e.g. tributyltin (TBT), or their release to the environment has significantly decreased, for example, air-transported heavy metals such as cadmium (Cd) or mercury (Hg), the high concentrations of legacy contaminants still influence the health of the marine environment (Löf et al., 2016a; HELCOM, 2018a).

In the case of legacy contaminants, the continuous negative impact stems from their slow degradation in the environment. Thus, this pool of contaminants exerts pressure on biota and habitats, including influence on genetic diversity, functional processes, biomass production, and the health and survival of higher trophic levels through biomagnification (Strand and Asmund, 2003; Viglino, et al., 2004; Cornelissen et al., 2008; Tansel et al., 2011; Li et al., 2021). In recent years, there has also been a remarkable shift in moving from the concept of high concentrations of a few contaminants in the marine environment to the understanding that in addition to these, there are many different pollutants that occur simultaneously and often in low concentrations (Rhind, 2009; Escher et al., 2020). With increasing knowledge of the toxic effects of this chemical “cocktail”, it has become apparent that the reliability of environmental assessments based on measurements of a relatively small number of selected substances may not be enough to understand the influence of various hazardous substances on marine biota and the functioning of ecosystems.

The main pathways of hazardous substances from highly industrialized and densely populated areas to the Baltic Sea are dominated by atmospheric deposition and riverine input (Pohl et al., 2006; Senze et al., 2015; Remeikaitė-Nikienė et al., 2018). The Baltic Sea is especially vulnerable to chemical pollution due to its limited water exchange and large catchment area (Schneider et al., 2000; Jędruch et al., 2017; HELCOM, 2021a). In recent decades, marine traffic, plus the expansion of existing, and development of new harbours has intensified, further increasing the risk of contamination (HELCOM, 2018b). For example, the high-intensity oil tanker traffic on the Baltic Sea has amplified pollution-associated issues such as waste disposal, accumulation of chemical compounds in sediments, and atmospheric deposition of toxic substances (Panov et al., 2002; HELCOM, 2018b). There are also other sources of concern, for example, renewable energy development, aquaculture, and dumped munitions, all of which may pose new pressures for the Baltic Sea ecosystem.

Based on the current knowledge, the hazardous substances causing the main threats to the Baltic Sea are Cu, Pb, Cd, Hg, TBT, PBDEs, and PAHs (HELCOM, 2023a). In general, these substances occur due to human activities, such as mining, use of synthetic pesticides, combustion of fossil fuels, waste incineration, production of different materials, pulp bleaching, and oil spills (Nor, 1987; Baumard et al., 1998; Martínez and Motto, 2000; Nour, 2019). Also, the Cu- or TBT-based antifouling paints on leisure boats and commercial ships are potential sources of those substances (Omae, 2003; Lagerström et al., 2017; Johansson et al., 2020).

1.2 Legislation related to the reduction of the negative impacts of hazardous substances on the Baltic Sea

A range of different policy or legislative initiatives are relevant for managing and monitoring the input of hazardous substances into the marine environment. Some of those being national, regional, some European, and some global in their perspective – though often these are also interlinked. These policies or legislation also differ in the level of legal enforcement and their specific focus area. At the regional level, the HELCOM Baltic Sea Action Plan (BSAP), updated in 2021, provides a vision for the Baltic Sea*:* "a healthy Baltic Sea environment with diverse biological components functioning in balance, resulting in a good ecological status and supporting a wide range of sustainable economic and social activities". As one of the core segments hazardous substances and marine litter are also addressed, with the goal of a "Baltic Sea unaffected by hazardous substances and litter". These segments are also supported by a range of ecological and management objectives as well as a number of more specific actions. At the global level, the United Nations Sustainable Development Goals and the Kunming-Montreal Global Biodiversity Framework are also significant players. These focus on the aims to "prevent and significantly reduce marine pollution of all kinds (Target 14.1)" and **"**reduce pollution to levels that are not harmful to biodiversity (Target 7)", respectively. Other relevant policy tools also support these broad aims, for example: the International Convention for the Prevention of Pollution from Ships (MARPOL) under the International Maritime Organisation (IMO), the Stockholm Convention, and the Minamata Convention.

In the European Union, several relevant regulatory and legislative tools exist. For example, the Water Framework Directive (WFD, European Commission 2000/60/EC, 2000), the Habitats Directive (European Commission 92/43/EEC, 1992), the EU Chemical Strategy (European Commission, 2020), the Urban Wastewater Directive, the European Green Deal, and the Registration, Evaluation, Authorisation and Restriction of Chemicals regulation (REACH (EC) 1907/2006) are all relevant. However, the Marine Strategy Framework Directive (MSFD) acts as a major driver of marine management activities, including in the Baltic Sea region. This forms the basis for jointly coordinated protection and monitoring of the marine environment with the main aim of establishing and maintaining good environmental status (GES; European Commission, 2008). The assessment of the marine environmental status is based on the descriptors listed in Annex I of the MSFD (European Commission 2008/56/EC, 2008), with further detailing of these in the Commission Decision (EU) 2017/848. One of the eleven descriptors, Descriptor 8 (D8), addresses the environmental pollution load with the broad aim of ensuring "concentrations of contaminants are at levels not giving rise to pollution effects" (Commission Decision (EU) 2017/848). In the Baltic Sea region, the Helsinki Commission (HELCOM) acts as a platform to support the Contracting Parties that are also EU Member States towards a coordinated implementation of the MSFD.

For evaluating the environmental status of the Baltic Sea based on the D8, threshold values (Table S1) for selected individual substances from different groups of contaminants (e.g., heavy metals, PAHs, PCBs, perfluorooctane sulphonate (PFOS), hexabromocyclododecane (HBCDD), polybrominated diphenyl ethers (PBDEs), BTs, pharmaceuticals, and radioactive substances) have been established through regional agreement, based on their toxic effect on aquatic biota (HELCOM, 2021b). Single substances are also included in the HELCOM indicator list mainly as core indicators and are used to evaluate the Baltic Sea's environmental status in holistic assessments[1]. Presently, indicators related to D8 Criteria 1 (D8C1) and designed with the concentration threshold values for selected contaminants in different matrices (water, sediment, and biota) are used as the main factor for the holistic assessment of the Baltic Sea's environmental status, these individual components being integrated into an overall summary (Fig. 1). At the same time, the biological effects caused by hazardous substances are only investigated based on a few indicators (HELCOM, 2010; 2018c; 2023a). Though, under MSFD D8, the assessment of biological effects caused by contaminants is defined in Criteria 2 (D8C2): preserving the health of the species and the condition of the habitats, with the assessment proposed to be based on factors such as community composition and species relative abundance.

[1] HELCOM Indicators: https://indicators.helcom.fi/ [accessed 13.05.2024]

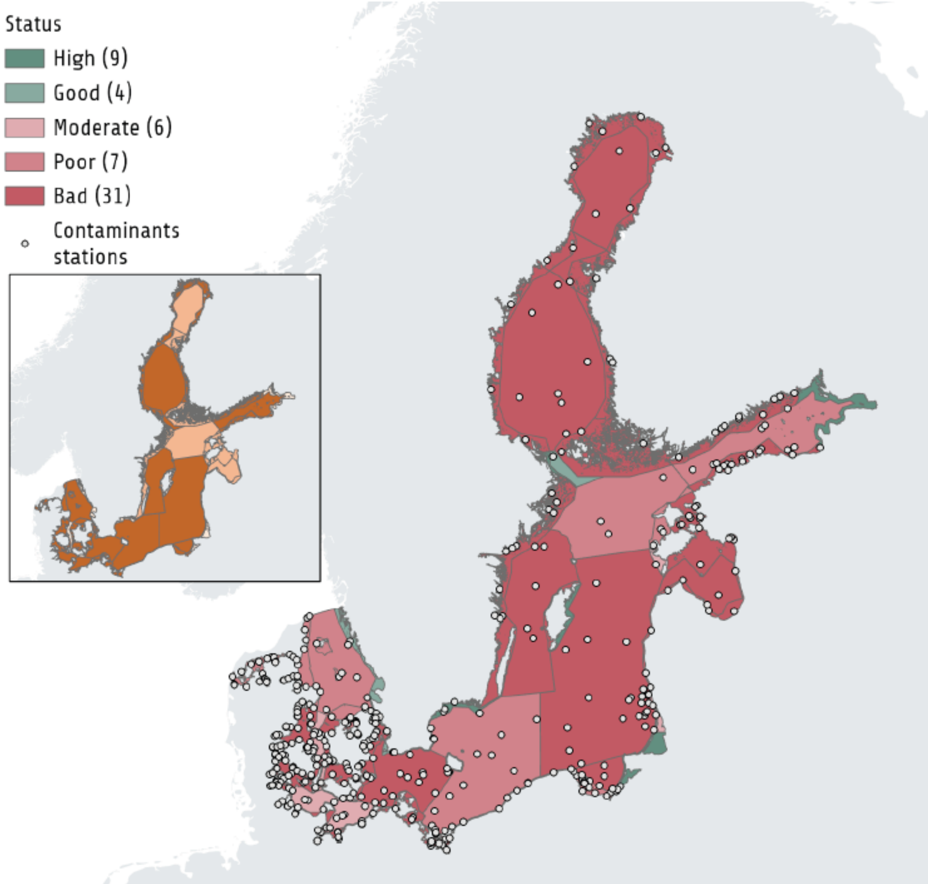

Figure 1. *Integrated Contamination Statement Assessment of the Baltic Sea. The red and green colours indicate the status of the Baltic Sea. The small map excerpt indicates the associated confidence in the assessment (per assessment unit) based on the underlying evaluation and data quality – darker shades representing higher confidence. The figure is adapted from the HELCOM HOLAS 3 assessment (2023).*

1.3 Biological effect indicators

Biological effect indicators facilitate quantification of the effect of contaminants on biota (Lam and Gray, 2003; Martín-Díaz et al., 2004). The influence of pollutants can be detected across different levels of biological organization, with measurable responses in animal biochemistry, physiology, reproduction, and behaviour (Sundelin et al., 2000; Lang and Wosniok, 2008; Gorokhova et al., 2010; 2020; Turja et al., 2014; Pérez and Hoang, 2017; Podlesińska and Dąbrowska, 2019; Berezina et al., 2022).

The establishment of quantifiable relationships between the pressures (contaminants and their mixtures) and biological effects is necessary for designing, selecting, and assessing environmental quality under D8C2, as well as for integrating the chemical and biological methods as indicators in the assessment (Queirós et al., 2016; Lyons et al., 2017). However, for the successful integration of biological effect methods, all confounding factors ideally have to be identified and accounted for, as much as possible, to guarantee that the threshold values and assessment criteria selected are ecologically meaningful (Gorokhova et al., 2010; Strode et al., 2023). Several environmental factors, such as sediment grain size, temperature, oxygenation, and total organic carbon content (TOC), are known drivers of the distribution and bioavailability of organic contaminants and metals in sediments (Tanner et al., 1993; Li et al., 2022). Thus, those variables need to be assessed for their potential contribution to the indicator variability in the specific region. Knowledge of the biological variability of the indicator species is also important to account for the potential differences resulting from the fluctuations in water temperature, dissolved oxygen concentrations, salinity, photoperiod, and food availability (Leiniö and Lehtonen, 2005; Barda et al., 2014; Braghirolli et al., 2016). Furthermore, the annual physiological cycle of organisms, such as reproductive status, may also cause variability in the indicator (Sheehan and Power, 1999; Braghirolli et al., 2016; Benito et al., 2019).

In the HELCOM framework, the main indicator development stages are candidate, pre-core, and core (HELCOM, 2020). These categories represent differing levels of development for a single indicator. Candidate and pre-core indicators are in preliminary stages and lack some components, or there is not yet a full agreement on their use among the Contracting Parties. For an indicator to be a HELCOM core indicator, all the Contracting Parties of the Helsinki Convention have to adopt the proposed indicator. For core indicators, monitoring is conducted and scientific evaluation is carried out against a well-defined, quantitative, and regionally approved threshold value(s) or environmental target(s) (HELCOM, 2020).

Supplementary indicators are similar to core indicators. However, they are only used by a few of the Contracting Parties in HELCOM, for example, due to the differences in the spatial coverage of species or pressure related to the proposed indicator, or for example where a lack of national monitoring may prevent application in some countries. Such supporting indicators provide more localized state or pressure evaluations or offer significant insights into relevant factors for interpreting other indicators and assessments, for example, characterizing important drivers, environmental factors, or actions across the Baltic Sea region (HELCOM, 2020).

The HELCOM indicator list currently contains three core indicators and one supplementary indicator related to the biological effects[1]. These provide information on contaminant exposure and its influence on biota. Although several pre-core indicators have been proposed earlier, there is a somewhat limited harmonised application of common indicators across the Baltic Sea region and several are not used by all the countries (Table 1). Based on this, the biological effects, as a stand-alone topic, especially focussing on single regionally agreed and regionally applied indicators, are not fully covered in HELCOM assessments, although some relevant components are evaluated based on the existing indicators (HELCOM, 2023a). Specific criteria have to be fulfilled for core indicator establishment, with data availability and approved threshold values being two critical components, as well as continuous input from national monitoring to support regular evaluations (HELCOM, 2020).

From the current HELCOM indicators[1], the indicator "White-tailed sea eagle productivity" is monitored in most of the countries surrounding the Baltic Sea that are part of the European Union (Table 1). However, the indicator is applicable for the assessment units close to the coast and does not reflect the effect on truly marine organisms. In addition, recent studies reveal that the main threats for the white-tailed eagle are mainly related to lead poisoning from ammunition while feeding on carcasses and also human-related accidents (Isomursu et al., 2018). The influence of contaminants on marine organisms is partially covered by the indicator “PAH metabolites in fish bile.” It reflects the level of exposure during the last few days before sampling, varying to some degree depending on the feeding activity of the fish[1]. This indicator, or sub-component of an indicator, is somewhat limited in its spatial application with only two countries collecting samples. Another indicator, “Imposex” evaluates the influence of elevated TBT concentrations on marine gastropods. However, this indicator is mainly utilized in the southern Baltic Sea (Table 1). At the same time, the “ReproIND” indicator (Reproductive disorders: malformed embryos of amphipods) is mainly applied in the northern subbasins (Table 1). The current situation allows for further improvement, for example, all of the mentioned indicators would benefit from longer time series data and increased spatial monitoring. However, one approach that can overcome the sub-regional differences in the application of individual indicators or variation in species distributions is to apply an integrated assessment, including different biological effect methods. A pilot study of such an approach was tested in the HOLAS 3 thematic assessment of hazardous substances, though it needs further development to include several aspects relevant to the marine environment assessment.

Table 1. *Biological effects monitored, their status as indicators, and use in different countries around the Baltic Sea. Modified from HELCOM (2020).*

Biological effect	**Current status of the indicator**	**SE**	**LV**	**DE**	**LT**	**DK**	**PL**	**EE**	**FI**
(TBT and) imposex	Core	Regular monitoring	Project	No	No	Regular monitoring	Pilot	No	No
(PAHs and) their metabolites	Core	No	No	Regular monitoring	No	No	Regular monitoring	No	No
White-tailed sea eagle productivity	Core	Regular monitoring	Regular monitoring	Regular monitoring	No	Project	Regular monitoring	Regular monitoring	Regular monitoring
Reproductive disorders: Malformed amphipod embryos	Supplementary	Regular monitoring	Project	No	No	Pilot	No	Project	No
Acetylcholinesterase inhibition (AchE)	Pre-core	Regular monitoring	Project	No	No	No	No	Project	No
Estrogenic-like chemicals and effects	Pre-core	Regular monitoring	No	No	No	No	No	No	No
Lysosomal membrane stability	Pre-core	Pilot	No	No	No	Regular monitoring	No	No	Regular monitoring
Fish disease index	Pre-core	Regular monitoring	No	Regular monitoring	No	No	Project	No	No
Micronucleus test	Pre-core	Pilot	No	Regular monitoring	No	No	Regular monitoring	No	Project
Ethoxyresorufin-O-deethylase activity (EROD)	Candidate	Regular monitoring	No	No	No	Regular monitoring	No	No	Project

1.3.1 Biological effect supplementary indicator "ReproIND"

One of the few biological effect indicators used for assessing the influence of contaminant impacts *in situ* is the Reproductive disorders: malformed embryos of amphipods (ReproIND) (HELCOM, 2023a; b). The indicator consists of two parameters: the proportion of aberrant embryos (%AbEmb) and the proportion of females with more than one aberrant embryo in their brood pouch (%Fem>1). When either of these parameters exceeds the established threshold, a good environmental status is not achieved. The indicator has been developed for two amphipod species, *Monoporeia affinis* Lindström, 1855 and *Pontoporeia femorata* Krøyer, 1842. However, it can also be applied to other amphipod species with similar reproductive history (Sundelin et al., 2008b; HELCOM, 2023d). Amphipods are regarded as well-suitable organisms for bioindication as they are widespread over different salinity and habitat ranges (Whiteley et al., 2011), and respond to various environmental contaminants (Turja et al., 2020). Different amphipod species have been widely used in sediment bioassay tests (Podlesińska and Dąbrowska, 2019). They are also ecologically relevant as they form an essential food source for many fish and other invertebrate species, which makes them suitable for ecotoxicological and food web-related studies (Podlesińska and Dąbrowska, 2019). In the Baltic Sea, the amphipod *M. affinis* is an ecological keystone species of the soft-bottom macrozoobenthic communities and, thus, a relevant bioindicator organism to monitor when evaluating anthropogenic influences (Sundelin and Eriksson, 1998; Lehtonen, 2004; Gorokhova et al., 2010; 2020).

The indicator offers insights into female exposure over an extended period of up to two years, encompassing growth, maturation, and oogenesis (Löf et al., 2016a). Several laboratory studies have reported the increased frequency of aberrant embryos in amphipods after contaminant exposure (Eriksson, et al., 1996; Sundelin et al., 2008a; Berezina et al., 2019, 2022). However, demonstrating the linkage between pollution and biological effects in the field is more challenging due to the simultaneous influence of several factors (McCarty and Mackay, 1993; Martín-Díaz et al., 2004). In the Baltic Sea, a higher frequency of aberrant embryos has been found in contaminated sites or the vicinity of contamination point sources compared to reference sites (Sundelin and Eriksson, 1998; Reutgard et al., 2014). In the *M. affinis* population, the frequency of females with aberrant embryos has demonstrated a positive correlation with elevated concentrations of Cd, PCBs, and PAHs in sediments (Löf et al., 2016a). However, the field evidence linking amphipod embryo aberrations to contaminants has been limited to sub-regional or local studies, with a restricted range of environmental factors and contaminant distribution considered (Reutgard et al., 2014; Berezina et al., 2016; Löf et al., 2016a; Strode et al., 2017). To effectively apply ReproIND in the environmental assessment of different Baltic subbasins, obtaining a broader geographical coverage of embryo aberration occurrence across diverse environmental settings is crucial.

Currently, the indicator has been utilized in the framework of the Swedish National Marine Monitoring Programme since 1994 for the assessment of the contaminant effects in Swedish coastal waters, the Bothnian Sea (BoS), The Quark, the Northern Baltic Proper (NBP), and the Western Gotland Basin (WGB; HELCOM, 2023d). Until the third HELCOM holistic assessment of the Baltic Sea (HOLAS 3, 2023), ReproIND had not been officially applied in the Gulf of Finland (GoF) and the Gulf of Riga (GoR). In Estonia, this indicator was first implemented in the GoF in 2016 using *M. affinis*. Later, the implementation was expanded to the GoR, and other amphipod species were also included.

1.4 Current gaps in knowledge on the distribution of contaminants and application of biological indicators

Environmental health assessments based on the concentrations and distribution of a small number of selected priority compounds provide only a portion of the information needed to understand the impact of hazardous substances on different organisms, their bioavailability, and the cumulative influence of hazardous substances on marine ecosystems. The focus on a small number of priority substances that are heavily monitored is important to understand the current situation and facilitate monitoring of inputs of contaminants to the marine environment. However, this approach is also being questioned as a stand-alone solution. This is due to increasing cumulative pressure from existing and emerging contaminants, present in the marine environment. This has led to the exploration of methodologies such as wide-scope screening and biological effects to help comprehend the issue. Additionally, to ensure compliance with D8 of the MSFD, there is a need for further development for monitoring biological effects. There is an urgent need to change the current strategy relying only on setting environmental quality standards and threshold values for single substances. There is accumulating scientific evidence that the pool of substances in the marine environment greatly exceeds the current list of priority substances and that contaminant mixtures often exceed the impact of a single substance, even in cases where substances individually appear to be at relatively low concentrations. While there is a need to improve the available data on the existing indicators, especially increasing the length of existing time series, further development of integrated approaches can bring together multiple individual methodologies into an overall assessment. This also contributes to approaches to link or compare the effects-based assessment outcomes with knowledge of *in situ* concentrations (i.e., a direct reflection of the pressure on biota).

1.5 Aims of the study

The aim of this PhD study was to aid the development of biological effect indicators to assess the impact of contaminants *in situ* and thus have a better understanding of the influence of contaminants on the organisms in the Baltic Sea.
This was achieved by:

- Generating an overview of the existing pressure by assessing the concentrations of contaminants in the different basins of the Baltic Sea (Paper I, II);
- Mapping the relationships between embryo aberrations in *M. affinis* and hazardous substances across several subbasins in the Baltic Sea and determining the potential effects of ecologically relevant confounding factors on the contaminant distribution and the identified reproductive responses in amphipods (Paper II);
- Validating the ReproIND indicator based on field data and expansion of its operationalization in the Baltic Sea (Paper II);
- Investigating seasonal variation in selected biochemical biomarkers and applying those for assessing amphipod communities in the Gulf of Riga (Paper III).

2. Material and methods

2.1 Sampling

Sampling was carried out in four subbasins of the Baltic Sea: the Bothnian Sea (BoS), the Western Gotland Basin (WGB), and the Gulfs of Finland (GoF) and Riga (GoR) during the period 2016–2021 (Fig. 2). The sediment samples used for analysing contaminants, total organic carbon (TOC), and grain size were collected from areas with different contamination levels. Those included relatively unpolluted regions (i.e., without known point sources) and sites close to intensive anthropogenic activities, e.g., next to ports, wastewater treatment plants, or sites with historical contamination from pulp or paper mills (Papers I, II).

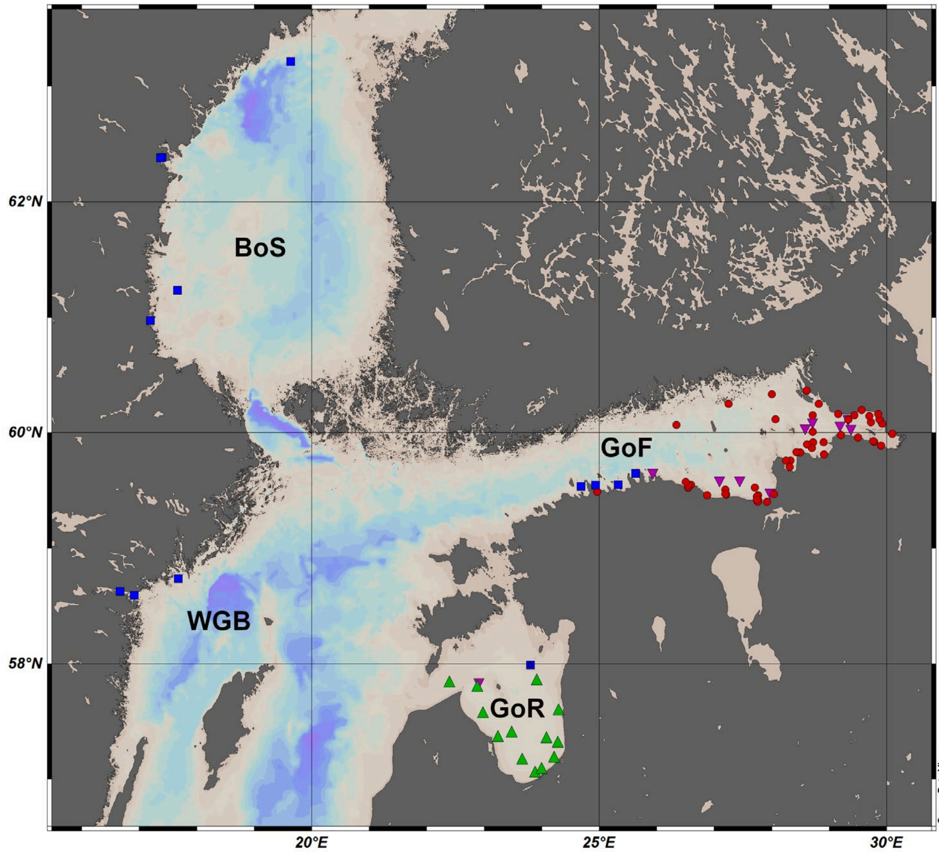

***Figure 2**. Sampling sites for Papers I-III. Filled red circles indicate stations investigated in Paper I; blue squares sites sampled in Paper II; green triangles in Paper III. Purple triangles indicate sites used in Paper I and Paper II; the green and purple triangles represent a station used in Paper II and Paper III.*

Amphipod samples for investigating embryo aberrations and biochemical biomarker analysis were collected with a bottom sled (Blomqvist and Lundgren, 1996) or with van Veen sediment grab (sample area of 0.1 m^2). Gravid *M. affinis* females for embryo aberration analysis were collected from 25 sites in four investigated subbasins from

December to March (Fig. 2). For biomarker analyses, *M. affinis* individuals were collected in August and November 2020 and 2021 during a cruise with R/V "Salme" (Fig. 2). Collected amphipods were immediately frozen in liquid nitrogen and later placed in a −80 °C freezer until biomarker analyses. Environmental parameters (e.g. dissolved oxygen, temperature, salinity) were also measured from a near-bottom layer.

2.2 Chemical and biological analyses

Chemical analyses from the collected sediments included heavy metals (arsenic (As), lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), mercury (Hg), zinc (Zn); mg kg^{-1}DW), polycyclic aromatic hydrocarbons (PAHs; ng g^{-1}DW), polychlorinated biphenyls (PCBs; ng g^{-1}DW), and bityltins (BTs; incl. tributyltin (TBT), dibutytin (DBT), and monobutyltin (MBT); ng g^{-1}DW). Sediment grain size (%) and total organic carbon (TOC, %) were also analysed. Analyses related to sediments were ordered from laboratories in Estonia, Russia, Latvia, Germany, and Sweden (Papers I, II).

Embryo analysis was conducted using a stereomicroscope following the methodology of Sundelin et al. (2008a). The number of analysed gravid females was recorded for each sampling site. In each female, fecundity (eggs per female), embryo development stage (from 1 to 9; Stage), and embryo aberrations following the classification by Löf et al. (2016a) were recorded (Paper II).

For analysing biomarkers (Paper III), whole bodies of five amphipods were pooled for each of the 12 replicate biomarker samples per station and homogenized for the measurements of enzyme activities of acetylcholinesterase (AChE; Ellman et al., 1961), catalase (CAT; Claiborne, 1985), glutathione S-transferase (GST; Habig *et al.*, 1974), and glutathione reductase (GR; Smith et al., 1988). The total amount of protein in the homogenate of each sample was determined with the Bradford assay using bovine serum albumin as the standard (Bradford, 1976).

2.3 Statistical analyses

In Paper I, the concentrations of some chemicals (anthracene, ANT, and TBT) were normalized to 5% TOC to facilitate comparison between sediments from different stations. In Paper II, log-transformed and normalized environmental and contaminant data were used for correlation and regression analyses and the resemblance matrices in the multivariate analysis.

Where relevant, the numerical variables were assessed for normality using the Shapiro-Wilk test (Papers II, III). Differences in sediment grain size (clay, sand, silt), TOC concentrations, and other environmental parameters (oxygen, salinity, and temperature) across the subbasins were evaluated using Kruskal-Wallis tests in R (R Core Team 2024; Paper II). In Paper III, a Kruskal–Wallis test, in combination with a Wilcoxon rank sum test, was used to compare seasonal and annual variability in medians of enzyme activities within and between the stations. In Paper II, the reproductive aberrations were analysed as frequencies (proportions), and the differences across the basins were evaluated using Fisher's exact test. In all studies (Papers I–III) the statistical significance level was set to $p < 0.05$.

In Paper II, multivariate ordination was applied to cluster the sampling sites based on their contaminant profiles and discover the environmental variables associated with contaminants. The missing data for contaminants was replaced using the maximum expectation likelihood algorithm with 1000 permutations implemented in Primer 7 v.7.0.21 (Anderson, 2017). FreeViz software was used for visualizing environmental, contaminant, and biological data (Demšar et al., 2007). A hierarchical cluster analysis was used to find the groups of sites that shared a similar composition and concentrations of contaminants. The classification was based on the Euclidean distance for similarity measures between sampling sites. Ward's method was used to establish links between the sites to improve the classification (Güler et al., 2002). After the clusters were identified, a comparison of reproductive aberration frequencies across these clusters was conducted using an unpaired t-test following Box-Cox transformation of the frequency data to stabilize variances.

Distance-based linear modelling was applied to evaluate the relative importance of environmental factors (sediment texture, temperature, oxygen, and TOC) in shaping the distribution of contaminants (concentrations and indices) as well as the influence of environmental factors and pollutants in explaining the observed reproductive responses in amphipods (Paper II). For this, subsets of variables were established using forward selection based on the multivariate analog to the small-sample-size corrected version of the Akaike Information Criterion corrected for small sample size (AICc). Relationships between environmental parameters and reproductive attributes were initially examined by analysing each predictor separately (marginal tests). Then, partial regressions were used to characterize the relationships accounting for the effect of the remaining variables by sequential tests with stepwise selection procedures and AICc as the selection criterion. The models were visualized using a distance-based Redundancy Analysis plot (dbRDA).

In Paper III, correlations between enzymatic biomarker levels and physicochemical variables were examined by using Spearman's rank correlation and visualized as a correlation matrix using the "corrplot" package (Wei and Simko, 2021) in R (R Core Team, 2024).

3. Results and discussion

3.1 Contaminants in the Baltic Sea

In general, the regulations banning the use of several hazardous substances and reducing chemical pollution have led to positive changes such as a decrease in the atmospheric and, to a lesser degree, in waterborne inputs of some metals and organic pollutants to the Baltic Sea (HELCOM, 2021c). However, the long-term reduction or at least stable situation in hazardous substances (three types of metals) has only been observed in a few countries, such as Germany, Finland, and Sweden, as those countries have the longest time series data available (HELCOM, 2021c).

Among other factors, long water exchange rates between the Baltic Sea and the North Sea enhance the entrapment of contaminants in the sediments (HELCOM, 2021a). Hazardous substances accumulate in fine-grained sediments rich in organic matter or that contain sulfides and iron-manganese hydroxides. They may be released from the sediments due to changing conditions of the seabed, such as changes in pH, dissolved oxygen, or temperature (Leivuori et al., 2000; Rigaud et al., 2013; Remeikaitė-Nikienė et al., 2018; HELCOM, 2021a). Thus, the different types of legacy contaminants are still present and biologically available in concentrations exceeding the established threshold values and limits set for the achievement of GES in different subbasins of the Baltic Sea (HELCOM, 2023a; Paper I). This is also visible from the results of the integrated contamination status assessment in the Baltic Sea region based on 11 core indicators, including 14 substances or substance groups and addressing different matrices, which showed that in the period 2016-2021, the GES was not achieved (HELCOM, 2023a).

The sediment samples collected within the framework of this thesis (Papers I, II) revealed that the highest contaminant loads were detected in the GoF, WGB, and BoS. In contrast, relatively low levels of all contaminants were present in the GoR. The elevated contaminant levels were associated with high salinity, low temperature, and fine-grained sediments (Fig. 3, Paper II), which has also been reported in previous studies (Höglund and Jonsson, 2008; Löf et al., 2016a; Erm, et al., 2021). In the majority of stations, the variability was mainly due to differences in the clay content (Fig. 3, Paper II). Among different contaminant groups, heavy metals were most widespread in all subbasins, whereas extremely high PAH levels were recorded in two stations in the BoS (SU57 and SU58). Those extreme values represent legacy contamination by pulp and paper mills operating in the area (Höglund and Jonsson, 2008; Apler et al., 2019). BTs were common in the Neva Estuary, particularly in the proximity to shipping lanes (e.g., sites 3F, 4F, 9F, and 2ug; GoF; Paper II)

More specifically, among metals, Cd concentrations in sediments exceeded the established threshold in 3% of measured sites (Supplementary Materials of Papers I, II). All sites with high concentrations of Cd were located in the Neva Estuary (GoF; Paper I, II). This result is somewhat inconsistent with the results of the HOLAS 3, where GES was achieved only in 29 of the 160 assessment units evaluated, based on Cd measurements in seawater, biota, and sediment (HELCOM, 2023c). For Cu (5% TOC normalized), concentrations were above the threshold in all investigated subbasins and 71% of the investigated sites, with the highest concentrations in BoS and GoF, 737.5 mg/kg DW and 525 mg/kg DW, respectively (Papers I, II). Based on previous results on the Cu concentrations in the Baltic Sea, GES was achieved only in one unit from the 24 evaluated (HELCOM, 2023d). It was found that the Pb concentrations (not normalized) were below threshold values in the sediments of all studied sites (Papers I, II). However, based on the HOLAS 3 results from three matrices (seawater, biota, sediment), GES was achieved only in 28 assessment units out of 159, mainly due to elevated concentrations in biota (HELCOM, 2023e).

In terms of TBT, high concentrations were detected in some of the sampling sites (Papers I, II), despite the regulations put in place regarding the use of TBT in most European countries more than decades ago (HELCOM, 2021a) and the international ban on using harmful organotins in antifouling paints since 2008 by the International Maritime Organization (IMO, 2008; Radke et al., 2008; Filipkowska et al., 2014; Remeikaitė-Nikienė et al., 2018; Jokšas et al., 2019). TBT (5% TOC normalized) concentrations were above the threshold in 40% of investigated sites and exceeded the threshold minimum 4.5% times (Papers I, II, Figs. 3, 4). In addition, a recent input of TBT was also detected (Paper I). The highest content of BTs was found in sediment samples across the GoF, especially in the Neva Estuary (Papers I, II, Figs. 3, 4). The HOLAS 3 report revealed that the GES was achieved only in one of the 83 assessment units based on the evaluation of TBT concentrations in seawater, sediments, and biological effects in marine gastropods (imposex) (HELCOM, 2023f).

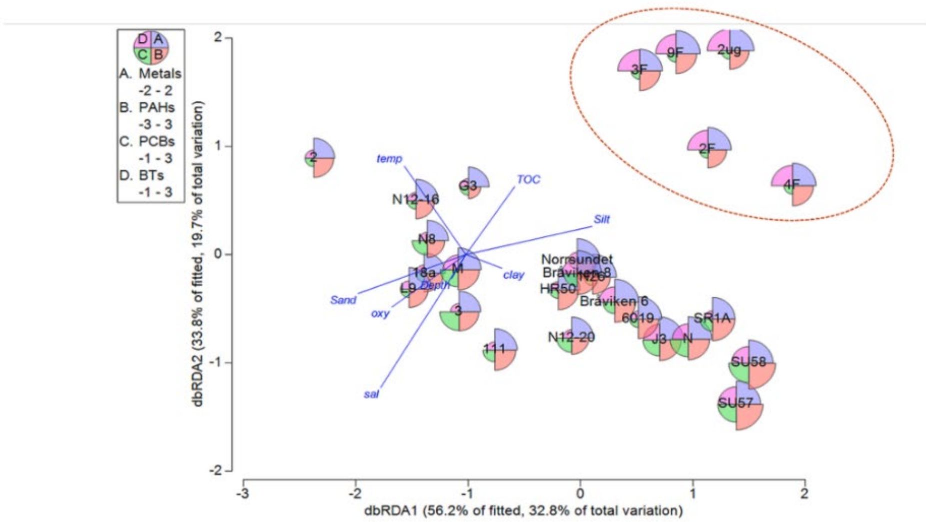

Figure 3. *dbRDA biplot for DistLM relating chemical load (by contaminant group, metals, PAHs, PCBs, and BTs) in sediment as multivariate response variables to environmental predictors: depth, temperature, oxygen, TOC, and sediment composition (clay, silt, and sand). The orange ellipse indicates stations located in the Neva Estuary (GoF). Modified from Paper II.*

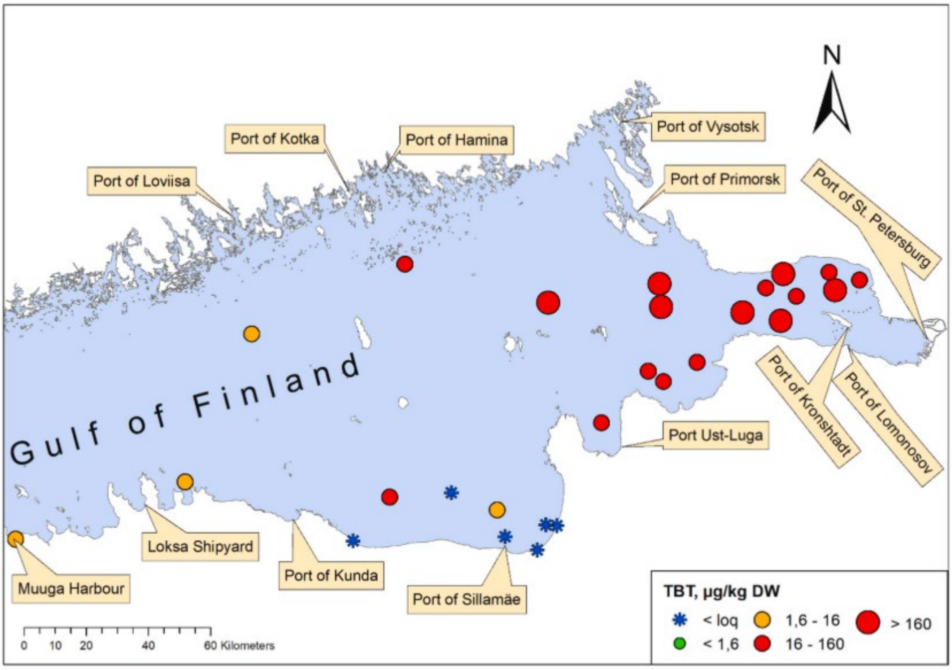

Figure 4. *Distribution of TBT (TOC-normalized) in sediments based on the data from 2019–2020. Orange spheres indicate values ranging from 1 to 10 times the HELCOM GES threshold until HOLAS 3 (1.6 µg/kg DW), red spheres: values ranging from 10 to 100 times the threshold, and large red spheres: values exceeding the threshold more than 100 times. Adapted from Paper I.*

For PAHs, the threshold values are established only for two congeners, anthracene, and fluoranthene. For these compounds, the threshold limits were exceeded in 20% and 4% of the measured samples (Papers I, II). For example, in the industrial area in BoS, concentrations of anthracene and fluoranthene exceeded threshold values approximately 940 and 32 times (Papers I, II). The PAHs achieved the threshold value in ten of the assessed 15 open sea and 73 of 95 coastal assessment units according to HOLAS 3 based on different matrices and compounds.

Finding genuinely unpolluted areas in the Baltic Sea may be challenging, even in areas given protection such as HELCOM Marine Protected Areas or Natura 2000 (Paper II, Fig. 5). This is in great part due to the potential for ubiquitous spread of hazardous substances once they enter marine or aquatic environments. Furthermore, it is important to consider the presence of a mixture of hundreds of hazardous substances in the natural environment, and evaluation of environmental status based on a few contaminants does not provide the complete picture of the actual situation. Thus, the current evaluation methods need to be combined with approaches that reflect the impact on the status of the biota. It should also be noted that actions to reduce concentrations of contaminants need to be continued by including a wider pool of substances.

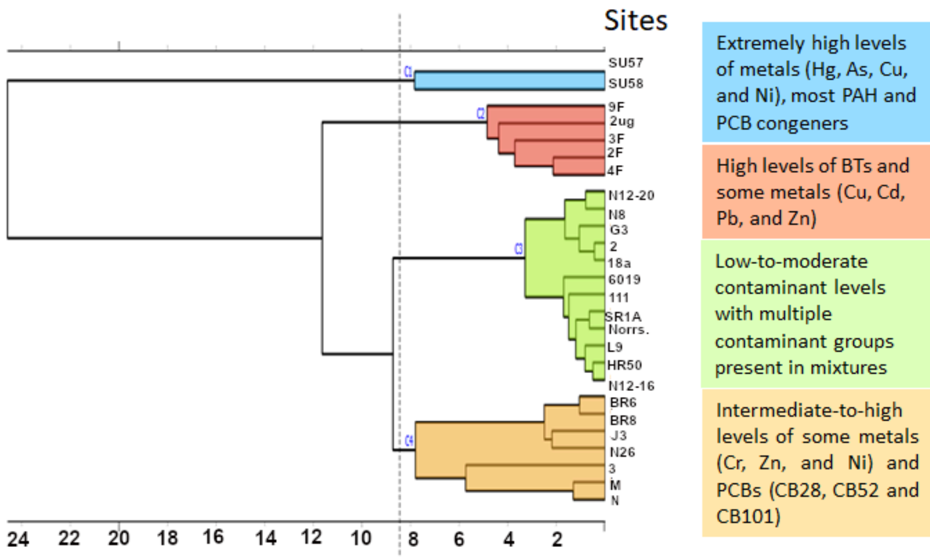

Figure 5. *Variability of the reproductive aberrations across sampling sites in Paper II grouped according to their contaminant load. Adapted from Paper II.*

3.2 Biological effect indicator: ReproIND

In the samples collected within this thesis, the embryo developmental stage and fecundity varied across basins (Paper II). The least developed embryos were present in the Neva Estuary, GoF. This is due to natural phenological variability across the subbasins and differences in the time of mating (December-January). The highest variability for the reproductive variables was detected in GoF, and the lowest in WGB (Paper II). Most of the GoF sites were characterized by relatively high levels of both %Fem > 1 and %AbEmb. However, a lower proportion of aberrant embryos was recorded in the Neva Estuary (Paper II).

When considering both chemical and non-chemical predictors, reproductive aberrations were significantly associated with environmental factors (salinity, temperature, TOC, and proportion of clay), PAH concentrations (naphthalene; NAP and dibenz(a,h)anthracene; DBAHA), and Pollution Load Index (PLI) as a proxy for metals (DistLM; Fig. 6). The environmental factors accounting for the differences between the subbasins explained most of the variability in the embryo aberrations, whereas individual contaminants explained up to 18 % of the captured variability. The aberration frequencies increased with the contaminant concentrations, temperature, and salinity and decreased in organic-rich sediments with a high proportion of fine (clay and silt) particles. An increase in embryo aberrations at higher temperatures aligns with the current knowledge of the ecology of *M. affinis* (Eriksson, et al., 2002; Wiklund and Sundelin, 2004). Furthermore, similar adverse effects of temperature on embryo development have been observed in other amphipod species in the region (Berezina et al., 2016).

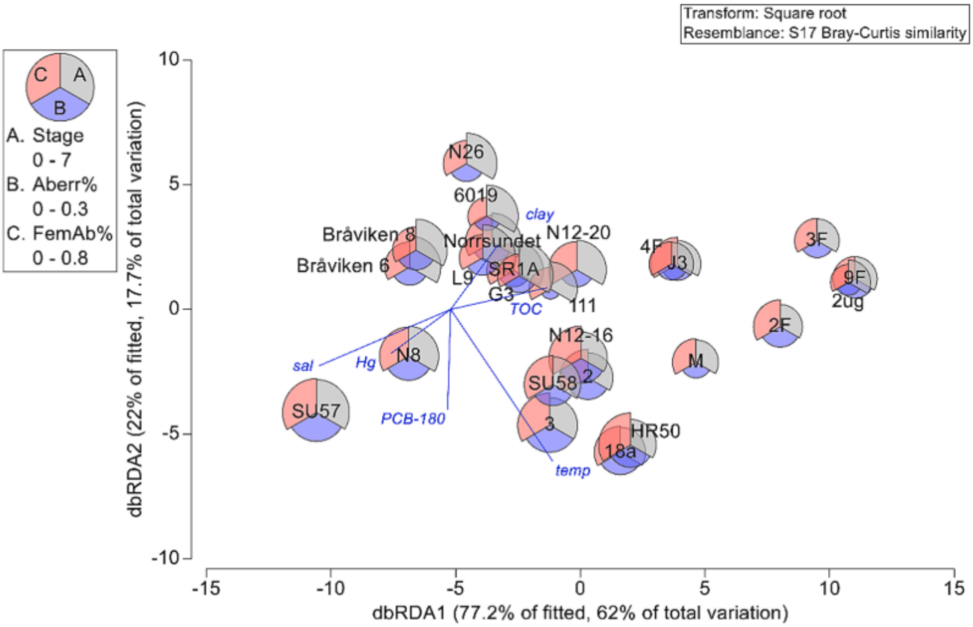

Figure 6. *Constrained ordination (dbRDA biplot) of the fitted values of the most parsimonious DistLM for reproductive aberration frequencies in amphipods (%AbEmb and %Fem > 1) as a multivariate response to the predictors identified by the model: contaminants (PCB-180 and Hg) and non-chemical environmental factors (temperature, TOC, salinity, and clay). Stage indicates the embryo developmental stage, adapted from Paper II.*

The significant increase of aberrations with increased contaminant concentrations was associated with metals (PLI, Hg), PAHs (NAP and DBAHA), and PCBs (PCB180). The metals (PLI, Hg), PAHs (NAP and DBAHA), and PCBs (PCB180, but also PCB28 and PCB118; Table 2 in Paper II) explained most of the variability in the reproductive aberrations (% AbEmb, %Fem > 1). These findings corroborate those of Löf et al. (2016a) from two localities in the Gulf of Bothnia, where the %AbEmb variability was best explained by PCBs (with PCB180 being the primary driver), PAHs (Phenanthrene, 1-Methylphenanthrene, benzo[ghi]perylene) and Cd. A higher aberration rate near known pollution sources has been reported as a general tendency in *M. affinis* and other amphipod species (Sundelin and Eriksson, 1998; Bach et al., 2010; Reutgard et al., 2014; Tairova and Strand, 2022). However, as contaminants occur in mixtures and under heterogeneous conditions, the univariate relationships between specific pollutants and reproductive responses should not be expected. This further motivates the development of biological indicators of contaminant exposure and effects that would be applicable across ecosystems.

From all the sampling sites, aberration frequency was relatively low in the Neva Estuary despite very high BT concentrations (Meador et al., 1997), coinciding with high TOC levels and clay/silt in the sediments (Fig. 6). High organic carbon content and fine-grained sediments have been reported to convey reduced contaminant bioavailability (Kreitinger et al., 2007; Baran et al., 2019). As hydrophobic BTs are absorbed into organic matter, their bioavailability is reduced (Rüdel, 2003; Cornelissen et al., 2005), leading to limited uptake and bioaccumulation as reported for tributyltin in the amphipod *Rhepoxynius abronius* J.L. Barnard, 1960 inhabiting high-TOC sediments (Meador et al., 1993; 1997). Moreover, interspecies variability in bioaccumulation and capacity to metabolize contaminants, such as TBT, as well as differences in the depth and rate of feeding, have been reported in amphipods (Byrén et al., 2002; Ohji et al., 2002). Further studies investigating intraspecies variability in *M. affinis* might explain the low embryo aberration rates in the Neva Estuary. The significant contribution of contaminants to the embryo aberration variability reported in Paper II supports the applicability of the ReproIND indicator in all Baltic Sea areas where *M. affinis* and several other amphipod species are present.

3.3 Enzymatic biomarkers in amphipods: response to seasonal variation

To expand the biological effect assessment and include non-reproductive responses, ReproIND can be combined with other biomarkers, e.g., antioxidant defense, geno- and cytotoxicity (Turja et al., 2020), metabolic activity, oxidative balance, neurotoxicity (Löf et al., 2016a;b), and DNA adductome (Gorokhova et al., 2020). However, it is essential to recognize the variability in the applied methods under different natural environmental conditions such as temperature, dissolved oxygen level, salinity, photoperiod, and food availability to facilitate the correct interpretation of biological responses to chemical contamination (Leiniö and Lehtonen, 2005; Barda et al., 2014; Braghirolli et al., 2016; Benito et al., 2019). Seasonal variability has been recognized as an important factor influencing the baseline levels of biochemical biomarkers and the organisms' responsiveness to pollution stress (Verlecar et al., 2008; Jemec et al., 2010; Braghirolli et al., 2016).

In this study (Paper III), seasonal variability was detected in all four analysed enzymatic biomarkers. The inhibition of AChE, a key enzyme in the cholinergic nervous system, showed less variability between two seasons compared to oxidative stress biomarkers CAT and GR and activity of glutathione GST (Fig. 7). In the case of CAT and GR, the variability between years was significant only from samples taken during November. In the previous studies from the Baltic Sea, significant seasonal differences have also been detected in the activity of enzymatic biomarkers in the clam *Macoma balthica* Linnaeus, 1758 from GoR (Barda et al., 2014) and GOF (Leiniö and Lehtonen, 2005).

Interestingly, AChE did not exhibit seasonal variability for *M. balthica* in GoR (Barda et al., 2014), whereas it was detected from the individuals collected from GoF and was explained by the differences in reproductive stage and main growth period of the species (Leiniö and Lehtonen, 2005). Furthermore, the result of the study from GoR showed that AChE inhibition could also be less variable compared to oxidative stress biomarkers due to seasonality. More multi-basin studies with different invertebrate species are needed to confirm this pattern. It could be suggested that oxidative stress biomarkers are more driven by changes in environmental variables, and their results should be assessed by considering the potential influence of season for each species separately.

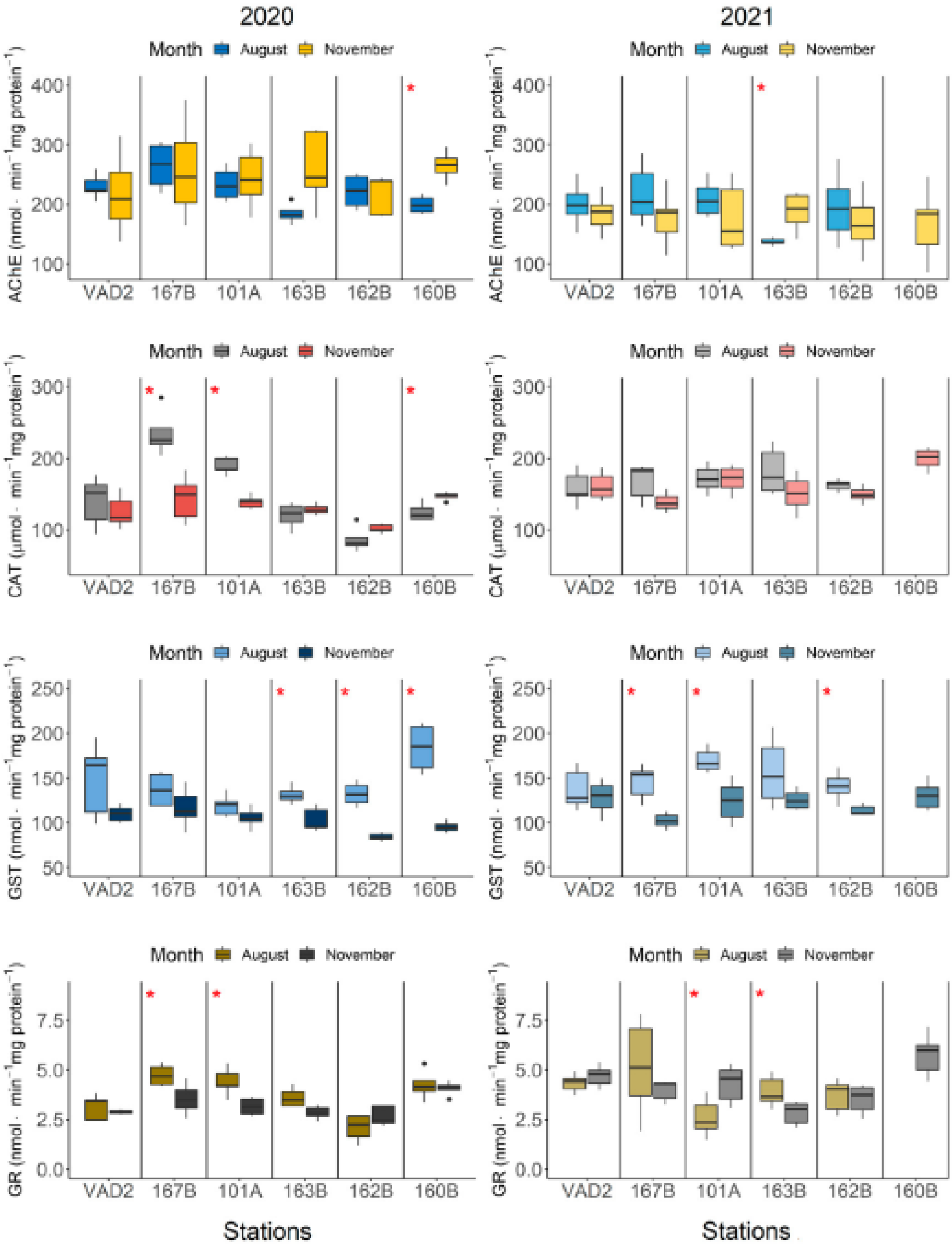

Figure 7. *The activity of AChE, CAT, GST, and GR in M. affinis at the coastal stations (20–30 m) in the GoR in August and November 2020 and 2021. The red asterisk indicates statistically significant (p < 0.05) seasonal differences at the respective stations. Filled circles (black) denote outliers. Modified from Paper III.*

3.4 Ongoing work and future perspectives

The HELCOM BSAP (HELCOM, 2021b) focuses on the actions toward achieving the GES of the Baltic Sea. One of the many actions listed in the BSAP segment "Hazardous substances and marine litter" is action HL13. This action aims to develop further relevant monitoring for the biological effects of hazardous substances to facilitate a reliable ecosystem health assessment by 2028. This necessitates the investigation of a more appropriate and ecologically relevant linkage of contaminant concentrations to biota and ecosystems. This aspect is also important for the MSFD D8C2. Currently, the members of the HELCOM Expert Group on Hazardous Substances have been successful in securing project funding to support this action, and pilot studies have been recently initiated. The projects include the Interreg-funded BEACON project, the Biodiversa+ project Detect2Protect, and the H-BEC project co-financed by the NEFCO Baltic Sea Action Plan Fund. These projects will also explore approaches to evaluate biological effects and how biological effects can be compared with and integrated with status outcomes from contaminant concentrations (HELCOM, 2023a).

For a long time, ReproIND was applied only by Sweden in a few of the Baltic Sea subbasins. By the study included in the thesis (Paper II), the application was confirmed for GoF and GoR; however, the indicator is yet to be included in the official monitoring programs of more countries. Thanks to the well-established methodology, scientific justification from laboratory and field studies, and its applicability to different amphipod species, the indicator has a good potential for application in other subbasins in the Baltic Sea to assess the effect of contaminants on biota. Denmark has already carried out the first steps and applied ReproIND in 2022 for shallow-water amphipods (Tairova and Strand, 2022; Tairova, et al., 2024).

In addition, the indicator could be combined with other biological effect methods (biochemical biomarkers, ethoxyresorufin-O-deethylase activity) and integrated into a multiapproach system. This approach would help to consider different methods used around the Baltic Sea on an equal footing, building them into a harmonised overview through careful fine-tuning of the system (e.g., the indicators would need calibrating properly to function together). An integrated approach employing several biological effects indicators would provide a better understanding of the broad impact of multiple mixed effects of contaminants (Turja et al., 2014; Lehtonen et al., 2014; 2016; Berezina et al., 2019) and support monitoring and management of environmental contaminants at the national, regional, and local scales.

Conclusions

Despite legislative efforts, contaminants in the marine environment are still present at levels above agreed threshold limits, and even fresh inputs of toxic substances banned decades ago still occur in the Baltic Sea. The data from this study reveals high concentrations of different types of hazardous substances in all of the investigated subbasins. For example, the concentrations of metals (Cd, Cu), TBT, and PAHs in the sediment exceeded the threshold limits regionally agreed through HELCOM. For example, high concentrations of TBT were found in GoF, especially in the Neva Estuary, and extremely high levels of PAHs were measured in BoS. Concentrations of Cu exceeded threshold limits in most studied sites in all subbasins. Our results broadly agree with the HOLAS 3 results, which show the failure to achieve GES in the case of hazardous substances in the Baltic Sea. For example, despite the worldwide ban on TBT use in antifouling paints for all types of boats in 2008, TBT persists as a major environmental pressure in the Baltic Sea.

Current monitoring programs are based mainly on the measurements of the concentrations of a small number of individual (or closely related groups) hazardous substances, whereas biological responses are given only limited consideration. However, the focus on a few substances is not representative of the real situation and methodologies to determine the toxicity of contaminants and their impacts on different levels of organisms are well established. *In situ* studies that consider contaminants and environmental factors together help to establish threshold values and develop scientifically confident monitoring of biological effects. Different biological effect methods have been developed and used to investigate the impact on biota as indicators for assessing the environmental status of the Baltic Sea. One such example is “ReproIND”. It provides information about the proportion of embryo aberrations and females carrying more than one aberrant embryo. This helps to estimate the reproductive success of amphipods in relation to the level of contamination. This study helped confirm the relation of “ReproIND” to sediment contamination and environmental factors *in situ* for four subbasins with an expansion of the indicator to GoF and GoR. The frequency of aberrations in the investigated amphipod species, *M. affinis,* was driven by both environmental factors (TOC and silt proportion, temperature, salinity) and hazardous substances (PAHs [DBAHA and NAP], PCB180, metals [PLI and Hg]). This agrees with previous studies reporting linkages between contaminant exposure and embryo aberrations.

“ReproIND” can be combined with other biological effect methods, such as enzymatic biomarkers, that can be measured in the same amphipod species. However, it has been found that enzymatic biomarkers demonstrate seasonal variability in dominant soft-bottom species (*M. affinis, M. balthica*) of the Baltic Sea. From the four investigated enzymes, AChE in *M. affinis* showed less seasonal variability. It could be suggested that this biomarker is more suitable for use in *M. affinis* as it is not influenced by seasonality. However, more studies are needed to confirm this for other basins.

This study supports the conclusion that biological effect methods can contribute significantly to our understanding of the Baltic Sea ecosystem health and that the approaches offer enough generality to be applied as a consortium to generate an integrated assessment approach for biological effects. To progress further the indicators must fulfill several criteria, such as further work towards agreed-upon threshold values and demonstrable links between pressures and effects. These would also need to be backed by more monitoring to maintain time series and expand spatial coverage. *In situ* studies that consider contaminants and environmental factors together support the development of scientifically validated monitoring of biological effects and contribute to policies directed toward achieving GES.

References

Anderson, 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef Stat. Ref. Online 1–15. https://doi.org/10.1002/9781118445112.stat07841

Antizar-Ladislao, B., 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. Environ. Int. 34, 292–308. https://doi.org/10.1016/j.envint.2007.09.005

Apler, A., Snowball, I., Frogner-Kockum, P., Josefsson, S., 2019. Distribution and dispersal of metals in contaminated fibrous sediments of industrial origin. Chemosphere 215, 470–481. https://doi.org/10.1016/j.chemosphere.2018.10.010

Bach, L., Fischer, A., Strand, J., 2010. Local anthropogenic contamination affects the fecundity and reproductive success of an Arctic amphipod. Mar. Ecol. Prog. Ser. 419, 121–128. https://doi.org/10.3354/meps08872

Baran, A., Mierzwa-Hersztek, M., Gondek, K., Tarnawski, M., Szara, M., Gorczyca, O., Koniarz, T., 2019. The influence of the quantity and quality of sediment organic matter on the potential mobility and toxicity of trace elements in bottom sediment. Environ. Geochem. Health 41, 2893–2910. https://doi.org/10.1007/s10653-019-00359-7

Barda, I., Purina, I., Rimsa, E., Balode, M., 2014. Seasonal dynamics of biomarkers in infaunal clam *Macoma balthica* from the Gulf of Riga (Baltic Sea). J. Mar. Syst. 129, 150–156. https://doi.org/10.1016/j.jmarsys.2013.05.006

Bashir, I., Lone, F.A., Bhat, R.A., Mir, S.A., Dar, Z.A., Dar, S.A., 2020. Concerns and Threats of Contamination on Aquatic Ecosystems BT - Bioremediation and Biotechnology: Sustainable Approaches to Pollution Degradation, in: Hakeem, K.R., Bhat, R.A., Qadri, H. (Eds.). Springer International Publishing, Cham, pp. 1–26. https://doi.org/10.1007/978-3-030-35691-0_1

Baumard, P., Budzinski, H., Michon, Q., Garrigues, P., Burgeot, T., Bellocq, J., 1998. Origin and Bioavailability of PAHs in the Mediterranean Sea from Mussel and Sediment Records. Estuar. Coast. Shelf Sci. 47, 77–90. https://doi.org/https://doi.org/10.1006/ecss.1998.0337

Benito, D., Ahvo, A., Nuutinen, J., Bilbao, D., Saenz, J., Etxebarria, N., Lekube, X., Izagirre, U., Lehtonen, K.K., Marigómez, I., Zaldibar, B., Soto, M., 2019. Influence of season-depending ecological variables on biomarker baseline levels in mussels (*Mytilus trossulus*) from two Baltic Sea subregions. Sci. Total Environ. 689, 1087–1103. https://doi.org/10.1016/j.scitotenv.2019.06.412

Berezina, N.A., Gubelit, Y.I., Polyak, Y.M., Sharov, A.N., Kudryavtseva, V.A., Lubimtsev, V.A., Petukhov, V.A., Shigaeva, T.D., 2016. An integrated approach to the assessment of the eastern Gulf of Finland health: A case study of coastal habitats. J. Mar. Syst. 171, 159–171. https://doi.org/10.1016/j.jmarsys.2016.08.013

Berezina, N.A., Lehtonen, K.K., Ahvo, A., 2019. Coupled Application of Antioxidant Defense Response and Embryo Development in Amphipod Crustaceans in the Assessment of Sediment Toxicity. Environ. Toxicol. Chem. 38, 2020–2031. https://doi.org/10.1002/etc.4516

Berezina, N.A., Sharov, A.N., Chernova, E.N., Malysheva, O.A., 2022. Effects of Diclofenac on the Reproductive Health, Respiratory Rate, Cardiac Activity, and Heat Tolerance of Aquatic Animals. Environ. Toxicol. Chem. 41, 677–686. https://doi.org/10.1002/etc.5278

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/https://doi.org/10.1016/0003-2697(76)90527-3

Braghirolli, F.M., Oliveira, M.R., Oliveira, G.T., 2016. Seasonal variability of metabolic markers and oxidative balance in freshwater amphipod *Hyalella kaingang* (Crustacea, Amphipoda). Ecotoxicol. Environ. Saf. 130, 177–184. https://doi.org/10.1016/j.ecoenv.2016.04.021

Byrén, L., Ejdung, G., Elmgren, R., 2002. Comparing rate and depth of feeding in benthic deposit-feeders: A test on two amphipods, *Monoporeia affinis* (Lindström) and *Pontoporeia femorata* Kröyer. J. Exp. Mar. Bio. Ecol. 281, 109–121. https://doi.org/10.1016/S0022-0981(02)00441-0

Castiglioni, S., Valsecchi, S., Polesello, S., Rusconi, M., Melis, M., Palmiotto, M., Manenti, A., Davoli, E., Zuccato, E., 2015. Sources and fate of perfluorinated compounds in the aqueous environment and in drinking water of a highly urbanized and industrialized area in Italy. J. Hazard. Mater. 282, 51–60. https://doi.org/10.1016/j.jhazmat.2014.06.007

Chaudry, M.A., Zwolsman, J.J.G., 2008. Seasonal dynamics of dissolved trace metals in the Scheldt estuary: Relationship with redox conditions and phytoplankton activity. Estuaries and Coasts 31, 430–443. https://doi.org/10.1007/s12237-007-9030-7

Claiborne, A., 1985. Catalase Activity. In: Greenwald, R.A., Ed., CRC Handbook of Methods for Oxygen Radical Research, CRC Press, Boca Raton, 283–284.

Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ. Sci. Technol. 39, 6881–6895. https://doi.org/10.1021/es050191b

Cornelissen, G., Pettersen, A., Nesse, E., Eek, E., Helland, A., Breedveld, G.D., 2008. The contribution of urban runoff to organic contaminant levels in harbour sediments near two Norwegian cities. Mar. Pollut. Bull. 56, 565–573. https://doi.org/10.1016/j.marpolbul.2007.12.009

Demšar, J., Leban, G., Zupan, B., 2007. FreeViz-An intelligent multivariate visualization approach to explorative analysis of biomedical data. J. Biomed. Inform. 40, 661–671. https://doi.org/10.1016/j.jbi.2007.03.010

Di Leonardo, R., Vizzini, S., Bellanca, A., Mazzola, A., 2009. Sedimentary record of anthropogenic contaminants (trace metals and PAHs) and organic matter in a Mediterranean coastal area (Gulf of Palermo, Italy). J. Mar. Syst. 78, 136–145. https://doi.org/10.1016/j.jmarsys.2009.04.004

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95. https://doi.org/https://doi.org/10.1016/0006-2952(61)90145-9

Eriksson, A.-K., Sundelin, B., Broman, D., Näf, C., 1996. Reproduction effect on *Monoporeia affinis* of HPLC-fractionated extracts of sediments from a pulp mill recipient., Environmental Fate and Effects of Pulp and Paper Mill Effluents.

Eriksson Wiklund, A.-K., Sundelin, B., 2002. Bioavailability of metals to the amphipod *Monoporeia affinis*: Interactions with authigenic sulfides in urban brackish-water and freshwater sediments. Environ. Toxicol. Chem. 21, 1219–1228. https://doi.org/https://doi.org/10.1002/etc.5620210615

Erm, A., Buschmann, F., Aan, A., 2021. Vertical distribution of priority substances and nutrients in the Strait Sea and Gulf of Riga. https://haldus.taltech.ee/sites/default/files/2021-02/KIK%2016300%20aruanne.pdf [In Estonian, accessed 20.05.2024]

Escher, B.I., Stapleton, H.M., Schymanski, E.L., 2020. Tracking complex mixtures of chemicals in our changing environment. Science 367, 388–392. https://doi.org/10.1126/science.aay6636

European Commission 2000/60/EC, 2000. Directive 2000/60/EC of European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. https://eur-lex.europa.eu/eli/dir/2000/60/oj [accessed 20.05.2024]

European Commission 2008/56/EC, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32008L0056 [accessed 20.05.2024]

European Commission 2017/848, 2017. Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017D0848 [accessed 20.05.24]

European Commission, 2020. Communication from the Commission to the European Parliament, the Council, the European Economic and the Committee of the Regions. Chemicals Strategy for Sustainability Towards a Toxic-Free Environment. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=COM%3A2020%3A667%3AFIN [accessed 20.05.2024]

European Commission 92/43/EEC, 1992. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A31992L0043 [accessed 20.05.2024]

Fattore, E., Bagnati, R., Colombo, A., Fanelli, R., Miniero, R., Brambilla, G., Di Domenico, A., Roncarati, A., Davoli, E., 2018. Perfuorooctane Sulfonate (PFOS), Perfluorooctanoic Acid (PFOA), Brominated Dioxins (PBDDs) and Furans (PBDFs) in wild and farmed organisms at different trophic levels in the Mediterranean Sea. Toxics 6, 1–7. https://doi.org/10.3390/toxics6030050

Filipkowska, A., Kowalewska, G., Pavoni, B., 2014. Organotin compounds in surface sediments of the Southern Baltic coastal zone: A study on the main factors for their accumulation and degradation. Environ. Sci. Pollut. Res. 21, 2077–2087. https://doi.org/10.1007/s11356-013-2115-x

Gorokhova, E., Löf, M., Halldórsson, H.P., Tjärnlund, U., Lindström, M., Elfwing, T., Sundelin, B., 2010. Single and combined effects of hypoxia and contaminated sediments on the amphipod *Monoporeia affinis* in laboratory toxicity bioassays based on multiple biomarkers. Aquat. Toxicol. 99, 263–274. https://doi.org/10.1016/j.aquatox.2010.05.005

Gorokhova, E., Martella, G., Motwani, N.H., Tretyakova, N.Y., Sundelin, B., Motwani, H. V., 2020. DNA epigenetic marks are linked to embryo aberrations in amphipods. Sci. Rep. 10, 1–11. https://doi.org/10.1038/s41598-020-57465-1

Güler, C., Thyne, G.D., McCray, J.E., Turner, A.K., 2002. Evaluation of graphical and multivariate statistical methods for classification of water chemistry data. Hydrogeol. J. 10, 455–474. https://doi.org/10.1007/s10040-002-0196-6

Habig, W.H.; Pabst, M.J.; Jakoby, W.B., 1974. Glutathione S-transferase. J. Biol. Chem. 249, 7130–7139. https://doi.org/10.1016/s0021-9258(19)42083-8

HELCOM, 2023a. HELCOM Thematic assessment of hazardous substances, marine litter, underwater noise and non-indigenous species 2016-2021. Balt. Sea Environ. Proceeding 190. https://helcom.fi/wp-content/uploads/2023/03/HELCOM-Thematic-assessment-of-hazardous-substances-marine-litter-underwater-noise-and-non-indigenous-species-2016-2021.pdf [accessed 20.05.2024]

HELCOM, 2023b. HELCOM Indicator Sheet: Reproductive disorders: malformed embryos of amphipods. https://helcom.fi/wp-content/uploads/2019/08/Reproductive-disorders-malformed-embryos-of-amphipods-HELCOM-supplementary-indicator-2018.pdf [accessed 20.05.2024]

HELCOM, 2023c. HELCOM Indicator Sheet: Cadmium. https://indicators.helcom.fi/indicator/cadmium/ [accessed 20.05.2024]

HELCOM, 2023d. HELCOM Indicator Sheet: Copper. https://indicators.helcom.fi/indicator/copper/ [accessed 20.05.2024]

HELCOM, 2023e. HELCOM Indicator Sheet: Lead. https://indicators.helcom.fi/indicator/lead/ [accessed 20.05.2024]

HELCOM, 2023f. HELCOM Indicator Sheet: Tributyltin (TBT) and imposex. https://indicators.helcom.fi/indicator/tbt-and-imposex/ [accessed 20.05.2024]

HELCOM, 2021a. Conditions that influence Good Environmental Status (GES) in the Baltic Sea. HELCOM ACTION. https://helcom.fi/wp-content/uploads/2021/11/Conditions-that-influence-Good-Environmental-Status-GES-in-the-Baltic-Sea.pdf [accessed 20.05.2024]

HELCOM, 2021b. HELCOM Baltic Sea Action Plan - 2021 update. https://helcom.fi/wp-content/uploads/2021/10/Baltic-Sea-Action-Plan-2021-update.pdf [accessed 20.05.2024]

HELCOM, 2021c. Inputs of hazardous substances to the Baltic Sea. Balt. Sea Environ. Proc. No. 179 1–27. https://helcom.fi/wp-content/uploads/2021/09/Inputs-of-hazardous-substances-to-the-Baltic-Sea.pdf [accessed 20.05.2024]

HELCOM, 2020. HELCOM Indicator Manual. Version 2020-1, Baltic Sea Environment Proceedings n° 175. https://repository.oceanbestpractices.org/bitstream/handle/11329/2320/BSEP175.pdf?sequence=1&isAllowed=y [accessed 20.05.2024]

HELCOM, 2018a. Inputs of hazardous substances to the Baltic Sea, Baltic Sea Environment Proceedings No. 162. https://helcom.fi/wp-content/uploads/2019/08/BSEP162.pdf [accessed 20.05.2024]

HELCOM, 2018b. Maritime activities in the Baltic Sea, Baltic Sea Environment Proceedings No. 152. https://www.helcom.fi/wp-content/uploads/2019/08/BSEP152-1.pdf [accessed 20.05.2024]

HELCOM, 2018c. State of the Baltic Sea- Second HELCOM Holistic Assessment 2011-2016, Baltic Sea Environment Proceedings 155. https://stateofthebalticsea.helcom.fi/wp-content/uploads/2018/07/HELCOM_State-of-the-Baltic-Sea_Second-HELCOM-holistic-assessment-2011-2016.pdf [accessed 20.05.2024]

HELCOM, 2010. Ecosystem Health of the Baltic Sea, Baltic Sea Environment Proceedings No. 122. https://helcom.fi/wp-content/uploads/2019/08/BSEP122.pdf [accessed 20.05.2024]

Höglund and Jonsson, 2008. Investigation concerning mercury barrels in Sundsvall Bay. https://viss.lansstyrelsen.se/ReferenceLibrary/51161/Utredning%20kring%20kvicksilvertunnor%20i%20Sundsvallsbukten_2008.pdf [In Swedish, accessed 20.05.2024]

IMO, 2008. International Convention on the Control of Harmful Anti-fouling Systems on Ships. https://www.imo.org/en/OurWork/Environment/Pages/Anti-fouling.aspx [accessed 20.05.2024]

Isomursu, M., Koivusaari, J., Stjernberg, T., Hirvelä-Koski, V., Venäläinen, E.R., 2018. Lead poisoning and other human-related factors cause significant mortality in white-tailed eagles. Ambio 47, 858–868. https://doi.org/10.1007/s13280-018-1052-9

Jędruch, A., Kwasigroch, U., Bełdowska, M., Kuliński, K., 2017. Mercury in suspended matter of the Gulf of Gdańsk: Origin, distribution and transport at the land–sea interface. Mar. Pollut. Bull. 118, 354–367. https://doi.org/10.1016/j.marpolbul.2017.03.019

Jemec, A., Drobne, D., Tišler, T., Sepčić, K., 2010. Biochemical biomarkers in environmental studies-lessons learnt from enzymes catalase, glutathione S-transferase and cholinesterase in two crustacean species. Environ. Sci. Pollut. Res. 17, 571–581. https://doi.org/10.1007/s11356-009-0112-x

Johansson, L., Ytreberg, E., Jalkanen, J.P., Fridell, E., Martin Eriksson, K., Lagerström, M., Maljutenko, I., Raudsepp, U., Fischer, V., Roth, E., 2020. Model for leisure boat activities and emissions - Implementation for the Baltic Sea. Ocean Sci. 16, 1143–1163. https://doi.org/10.5194/os-16-1143-2020

Johnston, E.L., Mayer-Pinto, M., Crowe, T.P., 2015. Chemical contaminant effects on marine ecosystem functioning. J. Appl. Ecol. 52, 140–149. https://doi.org/10.1111/1365-2664.12355

Jokšas, K., Stakėnienė, R., Raudonytė-Svirbutavičienė, E., 2019. On the effectiveness of tributyltin ban: Distribution and changes in butyltin concentrations over a 9-year period in Klaipėda Port, Lithuania. Ecotoxicol. Environ. Saf. 183. https://doi.org/10.1016/j.ecoenv.2019.109515

Knutzen, J., 1995. Effects on marine organisms from polycyclic aromatic hydrocarbons (PAH) and other constituents of waste water from aluminium smelters with examples from Norway. Sci. Total Environ. 163, 107–122. https://doi.org/https://doi.org/10.1016/0048-9697(95)04487-L

Kreitinger, J.P., Neuhauser, E.F., Doherty, F.G., Hawthorne, S.B., 2007. Greatly reduced bioavailability and toxicity of polycyclic aromatic hydrocarbons to Hyalella azteca in sediments from manufactured-gas plant sites. Environ. Toxicol. Chem. 26, 1146–1157. https://doi.org/10.1897/06-207R.1

Lagerström, M., Strand, J., Eklund, B., Ytreberg, E., 2017. Total tin and organotin speciation in historic layers of antifouling paint on leisure boat hulls. Environ. Pollut. 220, 1333–1341. https://doi.org/10.1016/j.envpol.2016.11.001

Lam, P.K.S., Gray, J.S., 2003. The use of biomarkers in environmental monitoring programmes. Mar. Pollut. Bull. 46, 182–186. https://doi.org/10.1016/S0025-326X(02)00449-6

Lang, T., Wosniok, W., 2008. The Fish Disease Index: a method to assess wild fish disease data in the context of marine environmental monitoring. ICES ACS. https://www.ices.dk/sites/pub/CM%20Doccuments/CM-2008/D/D0108.pdf [accessed 20.05.2024]

Langston, W.J., Pope, N.D., Davey, M., Langston, K.M., O’ Hara, S.C.M., Gibbs, P.E., Pascoe, P.L., 2015. Recovery from TBT pollution in English Channel environments: A problem solved? Mar. Pollut. Bull. 95, 551–564. https://doi.org/10.1016/j.marpolbul.2014.12.011

Lehtonen, K.K., 2004. Seasonal variations in the physiological condition of the benthic amphipods *Monoporeia affinis* and *Pontoporeia femorata* in the Gulf of Riga (Baltic Sea). Aquat. Ecol. 38, 441–456. https://doi.org/10.1023/B:AECO.0000035165.97619.78

Lehtonen, K.K., Sundelin, B., Lang, T., Strand, J., 2014. Development of tools for integrated monitoring and assessment of hazardous substances and their biological effects in the Baltic Sea. Ambio 43, 69–81. https://doi.org/10.1007/s13280-013-0478-3

Lehtonen, K.K., Turja, R., Budzinski, H., Devier, M.H., 2016. An integrated chemical-biological study using caged mussels (*Mytilus trossulus*) along a pollution gradient in the Archipelago Sea (SW Finland, Baltic Sea). Mar. Environ. Res. 119, 207–221. https://doi.org/10.1016/j.marenvres.2016.06.003

Leiniö, S., Lehtonen, K.K., 2005. Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 140, 408–421. https://doi.org/10.1016/j.cca.2005.04.005

Leivuori, M., Jokšas, K., Seisuma, Z., Kulikova, I., Petersell, V., Larsen, B., Pedersen, B., Floderus, S., 2000. Distribution of heavy metals in sediments of the Gulf of Riga, Baltic Sea. Boreal Environ. Res. 5, 165–185.

Li, Y., Zou, X., Zou, S., Li, P., Yang, Y., Wang, J., 2021. Pollution status and trophic transfer of polycyclic aromatic hydrocarbons in coral reef ecosystems of the South China Sea. ICES J. Mar. Sci. 78, 2053–2064. https://doi.org/10.1093/icesjms/fsab081

Li, Z., Zhang, W., Shan, B., 2022. Effects of organic matter on polycyclic aromatic hydrocarbons in riverine sediments affected by human activities. Sci. Total Environ. 815, 152570. https://doi.org/10.1016/j.scitotenv.2021.152570

Löf, M., Sundelin, B., Bandh, C., Gorokhova, E., 2016a. Embryo aberrations in the amphipod *Monoporeia affinis* as indicators of toxic pollutants in sediments: A field evaluation. Ecol. Indic. 60, 18–30. https://doi.org/10.1016/j.ecolind.2015.05.058

Löf, M., Sundelin, B., Liewenborg, B., Bandh, C., Broeg, K., Schatz, S., Gorokhova, E., 2016b. Biomarker-enhanced assessment of reproductive disorders in *Monoporeia affinis* exposed to contaminated sediment in the Baltic Sea. Ecol. Indic. 63, 187–195. https://doi.org/10.1016/j.ecolind.2015.11.024

Lyons, B.P., Bignell, J.P., Stentiford, G.D., Bolam, T.P.C., Rumney, H.S., Bersuder, P., Barber, J.L., Askem, C.E., Nicolaus, M.E.E., Maes, T., 2017. Determining Good Environmental Status under the Marine Strategy Framework Directive: Case study for descriptor 8 (chemical contaminants). Mar. Environ. Res. 124, 118–129. https://doi.org/10.1016/j.marenvres.2015.12.010

Marston, C.P., Pereira, C., Ferguson, J., Fischer, K., Hedstrom, O., Dashwood, W.M., Baird, W.M., 2001. Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumor initiation, PAH-DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. Carcinogenesis 22, 1077–1086. https://doi.org/10.1093/carcin/22.7.1077

Martín-Díaz, M.L., Blasco, J., Sales, D., DelValls, T.A., 2004. Biomarkers as tools to assess sediment quality: Laboratory and field surveys. TrAC Trends Anal. Chem. 23, 807–818. https://doi.org/10.1016/J.TRAC.2004.07.012

Martínez, C.E., Motto, H.L., 2000. Solubility of lead, zinc and copper added to mineral soils. Environ. Pollut. 107, 153–158. https://doi.org/10.1016/S0269-7491(99)00111-6

McCarty, L.S., Mackay, D., 1993. Enhancing Ecotoxicological Modeling and Assessment. Environ. Sci. Technol. 27, 1719–1728.

Meador, J.P., Krone, C.A., Wayne Dyer, D., Varanasi, U., 1997. Toxicity of sediment-associated tributyltin to infaunal invertebrates: Species comparison and the role of organic carbon. Mar. Environ. Res. 43, 219–241. https://doi.org/10.1016/0141-1136(96)00090-6

Meador, J.P., Varanasi, U., Krone, C.A., 1993. Differential sensitivity of marine infaunal amphipods to tributyltin. Mar. Biol. Int. J. Life Ocean. Coast. Waters 116, 231–239. https://doi.org/10.1007/BF00350012

Mearns, A.J., Reish, D.J., Oshida, P.S., Ginn, T., Rempel-Hester, M.A., Arthur, C., Rutherford, N., 2013. Effects of Pollution on Marine Organisms. Water Environ. Res. 85, 1828–1933. https://doi.org/10.2175/106143013x13698672322949

Nor, Y.M., 1987. Ecotoxicity of copper to aquatic biota: A review. Environ. Res. 43, 274–282. https://doi.org/https://doi.org/10.1016/S0013-9351(87)80078-6

Nour, H.E.S., 2019. Assessment of heavy metals contamination in surface sediments of Sabratha, Northwest Libya. Arab. J. Geosci. 12. https://doi.org/10.1007/s12517-019-4343-y

Ohji, M., Takeuchi, I., Takahashi, S., Tanabe, S., Miyazaki, N., 2002. Differences in the acute toxicities of tributyltin between the Caprellidea and the Gammaridea (Crustacea: Amphipoda). Mar. Pollut. Bull. 44, 16–24. https://doi.org/10.1016/S0025-326X(01)00146-1

Omae, I., 2003. Organotin antifouling paints and their alternatives. Appl. Organomet. Chem. 17, 81–105. https://doi.org/10.1002/aoc.396

Panov, V.E., Alimov, A.F., Golubkov, S.M., Orlova, M.I., Telesh, I. V, 2002. Environmental Problems and Challenges for Coastal Zone Management in the Neva Estuary (Eastern Gulf of Finland), in: Schernewski, G., Schiewer, U. (Eds.), Baltic Coastal Ecosystems: Structure, Function and Coastal Zone Management. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 171–184. https://doi.org/10.1007/978-3-662-04769-9_13

Pérez, E., Hoang, T.C., 2017. Chronic toxicity of binary-metal mixtures of cadmium and zinc to Daphnia magna. Environ. Toxicol. Chem. 36, 2739–2749. https://doi.org/10.1002/etc.3830

Podlesińska, W., Dąbrowska, H., 2019. Amphipods in estuarine and marine quality assessment – a review. Oceanologia 61, 179–196. https://doi.org/10.1016/j.oceano.2018.09.002

Pohl, C., Löffler, A., Schmidt, M., Seifert, T., 2006. A trace metal (Pb, Cd, Zn, Cu) balance for surface waters in the eastern Gotland Basin, Baltic Sea. J. Mar. Syst. 60, 381–395. https://doi.org/10.1016/j.jmarsys.2006.02.003

Queirós, A.M., Strong, J.A., Mazik, K., Carstensen, J., Bruun, J., Somerfield, P.J., Bruhn, A., Ciavatta, S., Flo, E., Bizsel, N., özaydinli, M., Chuševe, R., Muxika, I., Nygård, H., Papadopoulou, N., Pantazi, M., Krause-Jensen, D., 2016. An objective framework to test the quality of candidate indicators of good environmental status. Front. Mar. Sci. 3. https://doi.org/10.3389/fmars.2016.00073

R Core Team, 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.r-project.org/ [accessed 20.05.2024]

Radke, B., Łeczyński, L., Wasik, A., Namieśnik, J., Bolalek, J., 2008. The content of butyl- and phenyltin derivatives in the sediment from the Port of Gdansk. Chemosphere 73, 407–414. https://doi.org/10.1016/j.chemosphere.2008.05.020

Remeikaitė-Nikienė, N., Garnaga-Budrė, G., Lujanienė, G., Jokšas, K., Stankevičius, A., Malejevas, V., Barisevičiūtė, R., 2018. Distribution of metals and extent of contamination in sediments from the south-eastern Baltic Sea (Lithuanian zone). Oceanologia 60, 193–206. https://doi.org/10.1016/j.oceano.2017.11.001

Reutgard, M., Eriksson Wiklund, A.K., Breitholtz, M., Sundelin, B., 2014. Embryo development of the benthic amphipod *Monoporeia affinis* as a tool for monitoring and assessment of biological effects of contaminants in the field: A meta-analysis. Ecol. Indic. 36, 483–490. https://doi.org/10.1016/j.ecolind.2013.08.021

Rhind, S.M., 2009. Anthropogenic pollutants: A threat to ecosystem sustainability? Philos. Trans. R. Soc. B Biol. Sci. 364, 3391–3401. https://doi.org/10.1098/rstb.2009.0122

Rigaud, S., Radakovitch, O., Couture, R.M., Deflandre, B., Cossa, D., Garnier, C., Garnier, J.M., 2013. Mobility and fluxes of trace elements and nutrients at the sediment-water interface of a lagoon under contrasting water column oxygenation conditions. Appl. Geochemistry 31, 35–51. https://doi.org/10.1016/j.apgeochem.2012.12.003

Rocha, A.C.S., Reis-Henriques, M.A., Galhano, V., Ferreira, M., Guimarães, L., 2016. Toxicity of seven priority hazardous and noxious substances (HNSs) to marine organisms: Current status, knowledge gaps and recommendations for future research. Sci. Total Environ. 542, 728–749. https://doi.org/10.1016/j.scitotenv.2015.10.049

Rüdel, H., 2003. Case study: Bioavailability of tin and tin compounds. Ecotoxicol. Environ. Saf. 56, 180–189. https://doi.org/10.1016/S0147-6513(03)00061-7

Santhosh, K., Kamala, K., Ramasamy, P., Musthafa, M.S., Almujri, S.S., Asdaq, S.M.B., Sivaperumal, P., 2024. Unveiling the silent threat: Heavy metal toxicity devastating impact on aquatic organisms and DNA damage. Mar. Pollut. Bull. 200, 116139. https://doi.org/https://doi.org/10.1016/j.marpolbul.2024.116139

Schneider, B., Ceburnis, D., Marks, R., Munthe, J., Petersen, G., Sofiev, M., 2000. Atmospheric Pb and Cd input into the Baltic Sea: A new estimate based on measurements. Mar. Chem. 71, 297–307. https://doi.org/10.1016/S0304-4203(00)00058-X

Senze, M., Kowalska-Góralska, M., Pokorny, P., Dobicki, W., Polechoński, R., 2015. Accumulation of heavy metals in bottom sediments of Baltic sea catchment rivers affected by operations of petroleum and natural gas mines in western Pomerania, Poland. Polish J. Environ. Stud. 24, 2167–2175. https://doi.org/10.15244/pjoes/40273

Sheehan, D., Power, A., 1999. Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. Comp. Biochem. Physiol. - C Pharmacol. Toxicol. Endocrinol. 123, 193–199. https://doi.org/10.1016/S0742-8413(99)00033-X

Smith, I.K., Vierheller, T.L., Thorne, C.A., 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). Anal. Biochem. 175, 408–413. https://doi.org/https://doi.org/10.1016/0003-2697(88)90564-7

Staniszewska, M., Boniecka, H., Gajecka, A., 2013. Organochlorine, Organophosphoric and Organotin Contaminants, Aromatic and Aliphatic Hydrocarbons and Heavy Metals in Sediments of the Ports from the Polish Part of the Vistula Lagoon (Baltic Sea). Soil Sediment Contam. 22, 151–173. https://doi.org/10.1080/15320383.2013.722137

Stemmler, I., Lammel, G., 2009. Cycling of DDT in the global environment 1950-2002: World ocean returns the pollutant. Geophys. Res. Lett. 36, 1–5. https://doi.org/10.1029/2009GL041340

Strand, J., Asmund, G., 2003. Tributyltin accumulation and effects in marine molluscs from West Greenland. Environ. Pollut. 123, 31–37. https://doi.org/10.1016/S0269-7491(02)00361-5

Strode, E., Barda, I., Suhareva, N., Kolesova, N., Turja, R., Lehtonen, K.K., 2023. Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod *Monoporeia affinis* from the Gulf of Riga (Baltic Sea). Water 15, 248. https://doi.org/10.3390/w15020248

Strode, E., Jansons, M., Purina, I., Balode, M., Berezina, N.A., 2017. Sediment quality assessment using survival and embryo malformation tests in amphipod crustaceans: The Gulf of Riga, Baltic Sea AS case study. J. Mar. Syst. 172, 93–103. https://doi.org/10.1016/j.jmarsys.2017.03.010

Sumpter, J.P., 2009. Protecting aquatic organisms from chemicals: The harsh realities. Philos. Trans. R. Soc. A Math. Phys. Eng. Sci. 367, 3877–3894. https://doi.org/10.1098/rsta.2009.0106

Sunday, A.O., Alafara, B.A., Oladele, O.G., 2012. Toxicity and speciation analysis of organotin compounds. Chem. Speciat. Bioavailab. 24, 216–226. https://doi.org/10.3184/095422912X13491962881734

Sundelin, B., Eriksson, A.K., 1998. Malformations in embryos of the deposit-feeding amphipod *Monoporeia affinis* in the Baltic Sea. Mar. Ecol. Prog. Ser. 171, 165–180. https://doi.org/10.3354/meps171165

Sundelin, B., Rosa, R., Wiklund, A.K.E., 2008a. Reproduction disorders in the benthic amphipod *Monoporeia affinis*: An effect of low food resources. Aquat. Biol. 2, 179–190. https://doi.org/10.3354/ab00048

Sundelin, B., Ryk, C., Malmberg, G., 2000. Effects on the sexual maturation of the sediment-living amphipod *Monoporeia affinis*. Environ. Toxicol. 15, 518–526. https://doi.org/10.1002/1522-7278(2000)15:5<518::AID-TOX23>3.0.CO;2-K

Sundelin, B., Wiklund, A.K.E., Ford, A.T., 2008b. Biological effects of contaminants: the use of embryo aberrations in amphipod crustaceans for measuring effects of environmental stressors. ICES Tech. Mar. Environ. Sci. 41, 1–23.

Tairova, Z., J.P.A. Christensen, J.S., 2024. Reproductive disorders in amphipods as indicators of effects of hazardous substances in Danish waters. Aarhus University. https://dce.au.dk/fileadmin/dce.au.dk/Udgivelser/Notater_2024/N2024_10.pdf [accessed 20.05.2024]

Tairova, Z., Strand, J., 2022. Biological effect measurements in *Gammarus* spp. and *Corophium volutator* as indicators of toxic effects of hazardous substances in Danish coastal waters, Technical Report from DCE-Danish Centre for Environment and Energy. https://dce2.au.dk/pub/TR237.pdf [accessed 20.05.2024]

Tanner, P.A., James, J., Chan, K., Leong, L.S., 1993. Variations in trace metal and total organic carbon concentrations in marine sediments from Hong Kong. Environ. Technol. (United Kingdom) 14, 501–516. https://doi.org/10.1080/09593339309385320

Tansel, B., Fuentes, C., Sanchez, M., Predoi, K., Acevedo, M., 2011. Persistence profile of polyaromatic hydrocarbons in shallow and deep gulf waters and sediments: Effect of water temperature and sediment-water partitioning characteristics. Mar. Pollut. Bull. 62, 2659–2665. https://doi.org/10.1016/j.marpolbul.2011.09.026

Turja, R., Höher, N., Snoeijs, P., Baršiene, J., Butrimavičiene, L., Kuznetsova, T., Kholodkevich, S. V., Devier, M.H., Budzinski, H., Lehtonen, K.K., 2014. A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (*Mytilus trossulus*). Sci. Total Environ. 473–474, 398–409. https://doi.org/10.1016/j.scitotenv.2013.12.038

Turja, R., Sanni, S., Stankevičiūtė, M., Butrimavičienė, L., Devier, M.H., Budzinski, H., Lehtonen, K.K., 2020. Biomarker responses and accumulation of polycyclic aromatic hydrocarbons in *Mytilus trossulus* and *Gammarus oceanicus* during exposure to crude oil. Environ. Sci. Pollut. Res. 27, 15498–15514. https://doi.org/10.1007/s11356-020-07946-7

Verlecar, X.N., Jena, K.B., Chainy, G.B.N., 2008. Seasonal variation of oxidative biomarkers in gills and digestive gland of green-lipped mussel *Perna viridis* from Arabian Sea. Estuar. Coast. Shelf Sci. 76, 745–752. https://doi.org/10.1016/j.ecss.2007.08.002

Viglino, L., Pelletier, É., St.-Louis, R., 2004. Highly persistent butyltins in northern marine sediments: A long-term threat for the Saguenay Fjord (Canada). Environ. Toxicol. Chem. 23, 2673–2681. https://doi.org/10.1897/03-674

Wang, C., Du, J., Gao, X., Duan, Y., Sheng, Y., 2011. Chemical characterization of naturally weathered oil residues in the sediment from Yellow River Delta, China. Mar. Pollut. Bull. 62, 2469–2475. https://doi.org/10.1016/j.marpolbul.2011.08.021

Wang, X., Kong, L., Cheng, J., Zhao, D., Chen, H., Sun, R., Yang, W., Han, J., 2019. Distribution of butyltins at dredged material dumping sites around the coast of China and the potential ecological risk. Mar. Pollut. Bull. 138, 491–500. https://doi.org/10.1016/j.marpolbul.2018.11.043

Wei, T., Simko, V., 2021. R package "corrplot": Visualization of a Correlation Matrix. https://cran.r-project.org/web/packages/corrplot/corrplot.pdf [accessed 20.05.2024]

Whiteley, N.M., Rastrick, S.P.S., Lunt, D.H., Rock, J., 2011. Latitudinal variations in the physiology of marine gammarid amphipods. J. Exp. Mar. Bio. Ecol. 400, 70–77. https://doi.org/10.1016/j.jembe.2011.02.027

Wiklund, A.K.E., Sundelin, B., 2004. Biomarker sensitivity to temperature and hypoxia - A seven year field study. Mar. Ecol. Prog. Ser. 274, 209–214. https://doi.org/10.3354/meps274209

Yamazaki, E., Taniyasu, S., Ruan, Y., Wang, Q., Petrick, G., Tanhua, T., Gamo, T., Wang, X., Lam, P.K.S., Yamashita, N., 2019. Vertical distribution of perfluoroalkyl substances in water columns around the Japan sea and the Mediterranean Sea. Chemosphere 231, 487–494. https://doi.org/10.1016/j.chemosphere.2019.05.132

Yu, H., Lin, T., Hu, L., Lammel, G., Zhao, S., Sun, X., Wu, X., Guo, Z., 2023. Sources of polychlorinated biphenyls (PCBs) in sediments of the East China marginal seas: Role of unintentionally-produced PCBs. Environ. Pollut. 338, 122707. https://doi.org/10.1016/j.envpol.2023.122707

Zandaryaa, S., Frank-Kamenetsky, D., 2021. A source-to-sea approach to emerging pollutants in freshwater and oceans: pharmaceuticals in the Baltic Sea region. Water Int. 46, 195–210. https://doi.org/10.1080/02508060.2021.1889867

Acknowledgements

First and foremost, I would like to thank my supervisor, Sirje Sildever, whose encouragement was essential for completing this PhD journey. Your guidance, support, and patience have been invaluable.

I am also particularly grateful to Brita Sundelin, Elena Gorokhova, Evita Strode, and Ivan Kuprijanov, whose contributions have been instrumental to the success of this work. Your help and insights have been essential.

A special thanks to my family for their patience and constant support. Your encouragement has been a great source of strength for me.

Lastly, I want to thank my colleagues in the Department of Marine Systems at TalTech who believed in me and supported me along the way. Thank you all for being a part of this journey.

In its different phases, this work was supported by the funding from Environmental Investment Centre (KIK 10313, 16300, 17253, RE.4.07.22-0014), Estonia-Russia Cross-Border Cooperation Program 2014-2020 (HAZLESS project ER90), the Beacon project supported by the Interreg Baltic Sea Region and co-funded by the European Union (#S007 10/2022-10/2024), European Biodiversity Partnership Biodiversa + (D2P, Proposal number: 2021-473).

Abstract

Biological effects indicators: influence of environmental parameters and contaminants on amphipods

Despite the activities aimed at reducing the hazardous substances in the marine environment or banning certain substances, hazardous substances remain a significant problem. Hazardous substances enter the Baltic Sea mainly through rivers and the atmosphere and can persist in the marine environment for decades. They accumulate in sediments and pose a long-term threat to marine organisms. Currently, monitoring programs primarily use indicators based on determining concentrations of individual hazardous substances, but the impact of hazardous substances on biota is poorly assessed. One reason is the complexity of establishing quantifiable relationships between hazardous substances and biological effects in the environment, where other influencing factors are also present. Therefore, it is important to identify which environmental parameters and to what extent induce biological effects in marine organisms to account for this during the development of an indicator.

This study aimed to: **a)** provide an overview of the concentrations of hazardous substances in sediments and their variability in different basins of the Baltic Sea (Gulf of Finland, Gulf of Riga, Western Gotland Basin, Bothnian Sea) to demonstrate the persistence of the problem, **b)** expand the applicability of the biological effect indicator ReproIND (Reproductive disorders: malformed embryos of amphipods) to the Gulfs of Finland and Riga, using the amphipod species *Monoporeia affinis*, and **c)** investigate the use of enzymatic biomarkers to complement the ReproIND indicator. To achieve this, data on environmental parameters measured from the near-bottom layer during the period 2016–2021 (salinity, temperature, oxygen content), concentrations of hazardous substances in sediments, sediment grain size, and organic carbon content was collected analysed, along with data on embryo malformations from the four basins of the Baltic Sea. Enzymatic biomarker studies were conducted based on the samples originating from the Gulf of Riga during two seasons in 2020.

The concentrations of several hazardous substances in sediments exceeded the threshold values set for the Baltic Sea region. For example, copper concentrations exceeded the threshold values in all four subbasins and over 70% of the stations. Extremely high concentrations of polycyclic aromatic hydrocarbons (PAHs) were found from the stations in the Bothnian Sea and butyltins in the Gulf of Finland (the Neva Estuary). However, lead concentrations in sediments remained below the threshold value in all subbasins. Fresh additions of tributyltin to sediments were also detected, despite the ban of its use in anti-fouling paints since 2008. Those results demonstrate that the hazardous substances are widely distributed in the Baltic Sea in concentrations that pose a threat to marine organisms.

To assess the effects of hazardous substances on amphipods in the Baltic Sea, the ReproIND indicator was used. The indicator is based on the negative influence of hazardous substances on the embryos of *M. affinis*. The indicator consists of two parameters: the proportion of aberrant embryos and the proportion of females with at least one aberrant embryo in their brood pouch. When either of these parameters exceeds the established threshold, a good environmental status is not achieved. Recently, in the holistic assessment of the Baltic Sea published in 2023, the indicator was only used by Sweden. This allowed the application of the indicator only in a few

subbasins of the Baltic Sea. Further confirmation of the associations between concentrations of hazardous substances, environmental parameters, and reproductive disorders is necessary to develop and expand the indicator to other sub-basins. This provides a solid basis for expanding the indicator's applicability to other sub-basins and developing threshold values.

In this study, both hazardous substances and environmental parameters were found to contribute to the variability in embryo aberrations. For example, metals, PAHs, and polychlorinated biphenyls were positively associated with higher proportions of embryo malformations. From the environmental parameters, high temperatures and salinity, sandy sediments, and low organic carbon content were associated with a higher proportion of embryo aberrations. Those results are consistent with previous studies from the Baltic Sea region, confirming the usability of the indicator and demonstrating its applicability in the investigated sub-basins.

The ReproIND indicator can also be supplemented with other biological effect methods such as enzymatic biomarkers. Studies conducted in the Baltic Sea region show seasonal variability in enzymatic biomarker results among different dominant species. However, measurements of acetylcholinesterase (AChE) activity in *M. affinis* showed lower seasonal variability in the Gulf of Riga compared to oxidative stress biomarkers: glutathione S-transferase, glutathione reductase, and catalase. Previous studies from the same area on a different species, *Macoma balthica,* have shown similar results. Therefore, it can be assumed that AChE activity measurements can be applied regardless of the season. However, this needs confirmation by additional studies involving *in situ* measurements to confirm the relationships between enzymatic biomarker responses and hazardous substances. In the future, this would serve as a basis for developing the indicator and setting threshold values.

The next step to continue the development of biological effect indicators would be a revision of the threshold values calculated for the ReproIND indicator based on *M. affinis*. This would facilitate the assessment of the environmental status of the Gulfs of Finland and Riga within HOLAS 4, and the inclusion of the indicator in Estonia and Latvia's national marine monitoring programs. Since the indicator can also be based on other amphipod species, it is necessary to develop threshold values for amphipods living in shallow waters to enable the assessment of the coastal areas. Additionally, it is important to continue cooperation at the HELCOM region and on the European level to develop biological impact monitoring, encompassing various methods of biological impact assessment and complementing existing indicators of hazardous substances.

Lühikokkuvõte

Bioloogilise mõju indikaatorid: keskkonnaparameetrite ja ohtlike ainete mõju kirpvähilistele

Vaatamata tegevustele, mis on seotud ohtlike ainete kontsentratsioonide vähendamisega või teatud ainete keelustamisega, on ohtlikud ained merekeskkonnas jätkuvalt tähelepanu vajavaks probleemiks. Läänemerre sattuvad ohtlikud ained peamiselt jõgede ja atmosfääri kaudu ning võivad meres püsida aastakümneid. Ohtlikud ained akumuleeruvad meresetetesse ning nende järk-järgulisel vabanemisel võib mõju mereelustikule olla pikaajaline. Merekeskkonna seireprogrammides kasutatakse peamiselt üksikute ohtlike ainete kontsentratsioonidel põhinevaid indikaatoreid, kuid ohtlikest ainetest tingitud mõju elustikule hinnatakse vähe. Selle üks põhjus seisneb keerukuses tuvastada kvantitatiivseid seoseid ohtlike ainete ja bioloogiliste mõjude vahel keskkonnas, kus esineb ka teisi mõjutegureid. Seega on oluline välja selgitada millised keskkonnaparameetrid ja millisel määral võivad elustikku mõjutada, et seda indikaatori arendamisel arvesse võtta.

Antud töö eesmärkideks olid: **a)** kaardistada ohtlike ainete sisaldus setetes ja tuvastada nende varieeruvus erinevates Läänemere basseinides (Soome ja Liivi lahtedes, Gotlandi basseini lääneosas ja Botnia meres), **b)** laiendada bioloogilise mõju indikaatori ReproIND (Paljunemishäired: väärarenenud embrüod kirpvähilistel) rakendatavust Soome ja Liivi lahtedele, kasutades kirpvähilise liiki *Monoporeia affinis* ehk tavaline harjaslabalane ja **c)** uurida ensümaatiliste biomarkerite kasutamise võimalust ReproIND indikaatori täiendamiseks. Selleks kasutati perioodil 2016–2021 põhjalähedastes kihtides mõõdetud keskkonnaparameetrite (soolsus, temperatuur, hapniku sisaldus) andmeid, setetest määratud ohtlike ainete kontsentratsioone, setete lõimise analüüsi ja orgaanilise süsiniku sisaldust ning embrüote väärarengute andmeid neljast Läänemere basseinist. Ensümaatiliste biomarkerite uuringuid viidi läbi kahel erineval aastaajal 2020. aastal Liivi lahest kogutud proovide põhjal.

Mitmete mõõdetud ohtlike ainete kontsentratsioonid setetes ületasid Läänemere piirkonnale püstitatud piirväärtusi. Näiteks ületasid vase kontsentratsioonid piirväärtust kõigis neljas alambasseinis ja üle 70% jaamades. Ülikõrged polütsükliliste aromaatsete süsivesinike (PAHide) kontsentratsioonid mõõdeti Botnia merest pärinevatest setetest ja butüültina sisaldused Soome lahest, Neeva suudmealalt. Samas jäid plii kontsentratsioonid setetes alla piirväärtuse kõigis alambasseinides. Lisaks tuvastati ka jätkuv tributüültina lisandumine setetesse, vaatamata aine kasutamise keelustamisele kõikides laevade põhjavärvides alates 2008. aastast. See tähendab, et ohtlikud ained on Läänemeres laialt levinud kontsentratsioonides, mis on ohtlikud mereorganismidele.

Ohtlikest ainetest tingitud mõju hindamiseks kirpvähilistele on Läänemeres kasutusel indikaator ReproIND. On leitud, et ohtlike ainete poolt mõjutatud tavalise harjaslabalase emaste haudepaunas on rohkem väärarenenud embrüoid. Indikaator koosneb kahest parameetrist: väärarenenud embrüote osakaal ja emaste osakaal, mille haudepaunas on vähemalt üks arenenud embrüo. Juhul, kui vähemalt üks nendest parameetritest ületab püstitatud piirväärtust, ei ole hea keskkonna seisund saavutatud. 2023. aastal avaldatud Läänemere holistilises hindamise raporti põhjal oli indikaator kasutusel vaid Rootsis, mis võimaldab indikaatorit rakendada vaid üksikutes Läänemere alambasseinides. Indikaatori arendamiseks ja selle laiendamiseks teistele alambasseinidele on vajalik seoste kinnitamine ohtlike ainete sisalduste, keskkonnaparameetrite ja paljunemishäirete vahel.

Käesoleva töö käigus leiti, et embrüo väärarengute varieerumist mõjutavad nii ohtlikud ained kui ka keskkonnaparameetrid. Ohtlikest ainetest on embrüote väärarengutega seotud metallid, PAHid ning polüklooritud bifenüülid. Keskkonnaparameetritest on embrüote väärarengutega positiivselt seotud kõrged temperatuurid ja soolsus, liivased setted ja madal orgaanilise süsiniku kogusisaldus setetes. Saadud tulemus on kooskõlas varasemate Läänemere piirkonnas teostatud uuringutega, mis omakorda kinnitab indikaatori kasutatavust ja näitab selle rakendatavust ka teistes Läänemere basseinides.

ReproIND indikaatorit saab täiendada ka teiste bioloogiliste mõjude meetoditega nagu näiteks ensümaatilised biomarkerid. Läänemere piirkonnas teostatud uuringud näitavad, et erinevatel dominantliikidel esineb hooajaline muutlikus ensümaatiliste biomarkerite tulemustes. Liivi lahest kogutud *M. affinis*-e isenditga läbi viidud analüüside põhjal näitas kõige madalamat hooajalist varieeruvust atsetüülkoliinesteraasi (AChE) aktiivsus samas kui oksüdatiivse stressi biomarkerid (glutatiooni S-transferaas, glutatiooni reduktaas, katalaas) näitasid tulemustes suuremat hooajalist varieeruvust. Ka teise liigiga (*Macoma balthica*) samas piirkonnas läbi viidud uuringud näitasid sarnast tulemust. Seega võib eeldada, et AChE aktiivsuse näitaja sobib kasutamiseks sõltumata hooajast. Kuid kindlasti on vaja selle meetodi kasutuselevõtuks veel keskkonnas tehtud mõõtmisi, et kinnitada ensümaatiliste biomarkerite vastuste ning ohtlike ainete vahelist seost erinevates keskkonnatingimustes. See annab aluse indikaatori edasisele arendamisele ja piirväärtuste arvutamisele.

Järgmiseks sammuks on ReproIND indikaatori *M. affinis* jaoks arvutatud piirväärtuste ülevaatamine HOLAS 4 hindamiseks Soome ja Liivi lahtedes ning indikaatori lisamine Eesti ja Läti riiklikesse mereseire programmidesse. Kuna antud indikaatorit sobib kasutada ka teiste kirpvähiliste liikide põhjal on vaja arendada piirväärtusi ka madalas vees elavate liikide jaoks, et võimaldada rannikuala seisundi hindamist. Samuti on oluline jätkata nii HELCOM-i piirkonna kui ka Euroopa tasemel koostööd bioloogilise mõjude seire arendamiseks, mis hõlmaks erinevaid bioloogilise mõju meetodeid ja täiendaks olemasolevaid ohtlike ainete indikaatoreid.

Appendix 1

Publication I

Kuprijanov, I., Väli, G., Sharov, A., Berezina, N., Liblik, T., Lips, U., Kolesova, N., Maanio, J., Junttila, V., Lips, I., 2021. **Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland**. Mar. Pollut. Bull. 170. https://doi.org/10.1016/j.marpolbul.2021.112642

Marine Pollution Bulletin 170 (2021) 112642

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland

Ivan Kuprijanov [a,*], Germo Väli [a], Andrey Sharov [b], Nadezhda Berezina [c], Taavi Liblik [a], Urmas Lips [a], Natalja Kolesova [a], Jaakko Maanio [d], Ville Junttila [d], Inga Lips [a]

[a] Department of Marine Systems, Tallinn University of Technology (TalTech), Tallinn, Estonia
[b] Scientific Research Centre for Ecological Safety of the Russian Academy of Sciences (SRCES RAS), Saint Petersburg, Russia
[c] Zoological Institute of the Russian Academy of Sciences (ZIN RAS), Saint-Petersburg, Russia
[d] Finnish Environment Institute (SYKE), Helsinki, Finland

ARTICLE INFO

Keywords:
Organotins
PAHs
Heavy metals
Simulated accumulation
Baltic Sea

ABSTRACT

Contamination by hazardous substances is one of the main environmental problems in the eastern Gulf of Finland, Baltic Sea. A trilateral effort to sample and analyse heavy metals (HMs), polycyclic aromatic hydrocarbons (PAHs), and organotins from bottom sediments in 2019–2020 were conducted along with harvesting historical data in Russian, Estonian and Finnish waters. We suggest that the input of organotins still occurs along the ship traffic routes. The tributyltin content exceeded the established quality criteria up to more than 300 times. High contamination by PAHs found near the ports, most likely originate from incomplete fuel incineration processes. The Neva River Estuary and Luga Bay might potentially suffer from severe cadmium contamination. The high ecological risk attributed to the HMs was detected at deep offshore areas. The simulated accumulation pattern qualitatively agrees with field observations of HMs in sediments, demonstrating the potential of numerical tools to tackle the hazardous substances problems.

1. Introduction

Ecosystems of enclosed seas suffer from a multitude of stressors, an order of magnitude more than open ocean areas. Generally, cross-border environmental issues include eutrophication, the introduction of invasive species, and spreading of hazardous substances (HS). Concerns on the release of anthropogenic chemicals are already historically considered in the most serious manner as their human-induced occurrence is intimately linked with a direct impact on the health of biotic components in ecosystems. Trace and emerging pollutants became a matter of concern meanwhile after the advances of the chemical industry began to be associated with environmental degradation.

The global scale assessments of numerous potentially harmful chemicals revealed the scope of spread within aquatic ecosystems (e.g., Stemmler and Lammel, 2009; AMAP, 2017). Some hazardous substances were already banned (e.g., persistent organic pollutants as tributyltin (TBT)), or their release to the environment significantly decreased (e.g., air transported heavy metals as cadmium (Cd) or mercury (Hg)). Nevertheless, elevated concentrations of HS continue to affect the state of the environment (HELCOM, 2018a). Most of the chemical pollutants finally reach the sediments, which may develop into major reservoirs of these compounds (Fent, 2006; Rigaud et al., 2013). Contamination of the marine sediments is so far one of the main issues as accumulated pollutants infiltrate back to the water column through resuspension (Kalnejais et al., 2007) or enter the sediment-dwelling biota and accumulate along the trophic chains (Gilek et al., 1997; Blankholm et al., 2020; Nour, 2020).

There are many anthropogenic sources of heavy metals: the metal processing industry (mining, metal smelting), combustion of fossil fuels, use and recycling of certain products (e.g., batteries, plastics), disposal of waste and sewage water, application of fertilizers in agriculture, etc. (Rieuwerts et al., 1999; Komárek et al., 2008; Carolin et al., 2017; Nour, 2019). The main pathways of heavy metals from highly industrialized and densely populated areas to the Baltic Sea are mostly the atmospheric depositions and riverine input (Pohl et al., 2006; Senze et al., 2015; Remeikaitė-Nikienė et al., 2018). The riverine input of Cd and Pb to the Baltic Sea exceeds atmospheric deposition on average ca four times for Cd and two times for Pb (HELCOM, 2018f). Annual atmospheric

* Corresponding author.
E-mail address: ivan.kuprijanov@taltech.ee (I. Kuprijanov).

https://doi.org/10.1016/j.marpolbul.2021.112642
Received 31 January 2021; Received in revised form 7 June 2021; Accepted 15 June 2021
Available online 24 June 2021
0025-326X/

deposition of Cd and Pb to the Baltic Sea has dropped from 1990 to 2015 by 63% and 80%, respectively (Bartnicki et al., 2017), mainly due to strict regulations within the European Union (EU). Concentrations of Cd and Pb in water, biota and sediment were approved as the elements of a core indicator by HELCOM (The Baltic Marine Environment Protection Commission) and are in use for the Baltic Sea status assessment (HELCOM, 2018c).

The contamination by copper (Cu) is recognized as a significant concern for marine ecosystems (Dafforn et al., 2011). The detrimental effect of the element on the benthic organisms is confirmed by multiple studies (Stark et al., 2003; Xie et al., 2005; Han et al., 2008) though the toxicity of Cu is governed by the geochemical factors of the bottom sediments as well (Peng et al., 2004). The anthropogenic sources of Cu to the aquatic environment can be mining activities, use of synthetic pesticides and discharge from wastewater treatment plants (Nor, 1987; Martınez and Motto, 2000; Nour, 2019). In the Baltic Sea, the waterborne input from diffusive sources has the greatest contribution, with the highest load estimated for the Gulf of Finland (GoF) sub-region (HELCOM, 2011). The copper-based antifouling paints on leisure boats and commercial ships were recently estimated to be another significant source of Cu in the Baltic Sea (Johansson et al., 2020). Despite the long-term active monitoring, established regulations at national levels (KEMI, 2006; Sahlin and Ågerstrand, 2018) and existing environmental quality standards (Long et al., 1995; Canadian Council of Ministers of the Environment, 2002), Cu does currently not belong to the HELCOM indicators.

The organotin compounds (OTCs) in the marine environment originate mainly from antifouling paints (Viglino et al., 2004; Radke et al., 2008). TBT-based antifouling paints were used since the 1960s to protect ship hulls from biofouling by algae and invertebrates (Omae, 2003; Lagerström et al., 2017). In addition, OTCs were widely used as biocides in agriculture, plastic stabilizers, and wood preservation (Hoch, 2001). TBT is highly toxic for aquatic invertebrates and causes adverse effects even in low concentrations (Antizar-Ladislao, 2008; Sunday et al., 2012). Though, in the aquatic environment, TBT can be rapidly degraded by biological activity and photomediated reactions to the less toxic by-products such as monobutyltin (MBT) or dibutyltin (DBT) (Rodriguez-Gonzalez et al., 2013; Rodriguez-Cea et al., 2016). OTCs can persist in sediments in the range of years (de Mora and Pelletier, 1997; Cornelissen et al., 2008) and days or weeks in the water (Lee et al., 2006; Rodriguez-Gonzalez et al., 2013).

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants, which are mainly introduced to the environment by human activities, became ubiquitous in the coastal zones (de Boer et al., 2001). The oil spills and emission of exhaust particles during incomplete combustion of fossil fuels are predominantly responsible for the release of these substances (Baumard et al., 1998; Neff, 2004). The determination of 16 U.S. Environmental Protection Agency (EPA) priority PAHs is the widespread practice in the monitoring of the aquatic environment (Keith, 2015; McGrath et al., 2019). The detrimental effect of chemicals is known to be compound-specific, though the synergistic effect of several PAH congeners might be larger (Knutzen, 1995; Marston et al., 2001). The mixtures of PAHs after deposition into environmental matrices comprise the low and high molecular weight PAHs. Depending on the number of aromatic rings, the low molecular weight compounds as anthracene or naphthalene are shown to be acutely toxic for marine organisms, while the high molecular weight PAHs (e.g., benzo(*a*)pyrene, dibenzo(*a*,*h*)anthracene, benzo(*b*)fluoranthene) are less toxic and more associated with chronic effects, i.e. carcinogenicity (Menzie et al., 1992; Kennish, 1997). The contamination assessment of marine sediments in the Baltic Sea by PAHs is set to be conducted on the evaluation of the priority pollutant, anthracene, however, its distribution is not monitored sufficiently in the eastern parts of the Baltic Sea, specifically, data from the Gulf of Finland is very scarce (HELCOM, 2018e).

The Gulf of Finland is a shallow 48–125 km wide sub-basin in the north-eastern Baltic Sea with an average depth of 35 m. It has a free water exchange with the open Baltic Sea and its water residence time is less than two years (Alenius et al., 1998; Liblik and Lips, 2017). The Gulf of Finland is characterized by strong water column stratification (e.g., Liblik and Lips, 2017) and excess of nutrients (e.g., Pitkänen et al., 2001) that in turn facilitate the development of wide-scale hypoxia events at the bottom environment (e.g., Stoicescu et al., 2019). Human-induced eutrophication has led to an increase in hypoxia in the Baltic Sea coastal zone (Conley et al., 2011). During the last decades, drastically intensified marine traffic and vigorous development of new harbour areas transformed the Baltic Sea into a waterbody with highly expanded international maritime activity (HELCOM, 2018b). Immense oil tanker traffic intensity (HELCOM, 2010) exacerbated already known issues related to pollution associated with the disposal of industrial and domestic waste, along with atmospheric deposition of toxic compounds, which all became the main environmental concern addressed in the eastern Gulf of Finland (Panov et al., 2002; Ryabchuk et al., 2017). Although, despite the lack of data on some persistent HS in the eastern Gulf of Finland, it is potentially one of the most polluted areas in the Baltic Sea as revealed by human pressures mapping (Korpinen et al., 2012).

Countries on the Baltic coast traditionally have a far from equal situation regarding the occurrence of HS and assessment of their distribution, which in turn is mirrored in differences of national monitoring programs. However, several compounds cause troubles regardless of the sea region. The HELCOM indicators are agreed to be the comprehensive measures of environmental health. The calculated indices help recognize the degree of contamination by heavy metals (Hakanson, 1980; Nour and Nouh, 2020), sources, in case of contamination by polycyclic aromatic hydrocarbons (PAHs) (Khairy et al., 2009), or estimate the fate of the pollutant by its degradation products, widely applied for TBT (Díez et al., 2002; Filipkowska et al., 2014).

Our aim was to address the existing gaps in the studies of priority substances, included in the HELCOM Core Indicators list (e.g., heavy metals, PAHs, TBT; HELCOM, 2013), in the bottom sediments of the eastern Gulf of Finland. Within the framework of our study, the trilateral collection of the HS data in Russia, Estonia and Finland in 2019–2020 and compilation of available older data were conducted. The present cross-border study will contribute to the reliable assessment of HS in different matrixes in the eastern Gulf of Finland. We differentiate the sources of PAHs and suggest the temporal extent of contamination events of the TBT and determine the potential degree of the risk the heavy metals might pose in the study area according to the calculated indices. Likewise, we aim to estimate the pathways of HS by applying numerical modelling optimized for the conditions of the study area.

2. Methods

2.1. Dataset

The sediment samples for chemical analyses of HS were collected in the Russian and Estonian territorial waters in 2019–2020 (Fig. 1). In addition, the dataset of HS measured in the upper layer of marine sediments during previous monitoring (including national monitoring) and research activities in the area has been compiled. Data on HS in sediments cover the years from 2010 to 2017 (obtained from Estonian national database "KESE" and database from TalTech Department of Marine Systems) and from 2005 to 2018 (obtained from published sources and databases of Russian research institutions). Monitoring data collected by Finnish Environment Institute (Helsinki, Finland) was extracted from the ICES database and comprised of two monitoring stations visited in 2017–2019 (ICES DOME dataset).

Chemical parameters from the HELCOM indicator list (HELCOM, 2018a) relevant for the sediment environment were selected for the data collection in 2019–2020. Substances with defined threshold values for the Baltic Sea included potentially harmful trace metals (Pb, Cd), persistent organic pollutant (TBT) and priority pollutant PAH compound

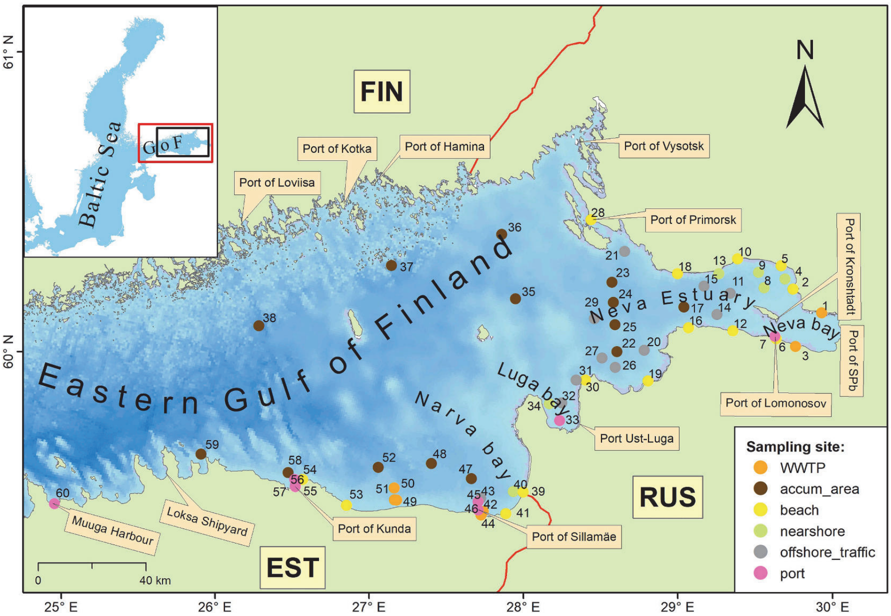

Fig. 1. Study region, map of sediment sampling locations during 2019–2020 fieldworks, the classification of sampling sites is presented by coloured dots (WWTP - vicinity of wastewater treatment plant, accum_area - sediment accumulation area, beach - shallow coastal beach area, nearshore - area near the shore, offshore_traffic - the route of heavy marine traffic, port - area next to port; Table S5). Red frame around the Gulf of Finland on the small panel of the Baltic Sea indicates the extent of the domain of the high-resolution nested model setup. The names of the stations corresponding to the numerical labels on map can be found in the Table S5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

anthracene (Table 1). From 35 stations, where organotins were measured, at seven stations, only TBT was analysed, and at 28 stations, concentrations of the five cations (MBT, DBT, TBT, monooctyltin, and triphenyltin) were analysed. Additionally, tetrabutyltin was analysed at 26 stations, triphenyltin and monophenyltin at 13 stations, while methyltin-cations were determined at 15 stations only from the Russian part of the study area. The organo-metallic pollutants were aggregated as the sum of organotin compounds (OTCs), the sum of butyltins (BTs) comprised of MBT, DBT, TBT and tetrabutyltin.

The 16 PAHs analysed from sediment samples collected at 52 stations are recommended as priority pollutants by the U.S. EPA, EU Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD) (HELCOM, 2012). The abbreviations used for the PAHs are following: acenaphthene (ACNE), acenaphthylene (ACNLE), anthracene (ANT), benz(*a*)anthracene (BAA), benzo(*a*)pyrene (BAP), benzo(*b*)fluoranthene (BBF), benzo(*g,h,i*)perylene (BGHIP), benzo(*k*)fluoranthene (BKF), chrysene (CHR), dibenz(*ah*)anthracene (DBAHA), fluoranthene (FLU), fluorene (FLE), indeno(1,2,3-*cd*)pyrene (ICDP), naphthalene (NAP), phenanthrene (PA), pyrene (PYR). Trace metals Cd, Pb, Cu and zinc (Zn) were analysed from 56 sampling stations.

For all the samples from Estonian territorial waters, the organic content and granulometric analysis of sediment were performed. The laser diffraction method combined with sieving was applied for the determination of the grain size of the bottom sediment samples. The percentage of fine-grained (<63 μm) and coarser sediment fractions were evaluated. Total organic carbon (TOC) content was determined according to the wet oxidation method (Tjurin's method), by registering the amount of carbon dioxide released (Arinushkina, 1970) or by dry combustion acc. to DIN EN 15936 analytical standards. The TOC content of sediment samples from 8 stations (Letipea, Koh_wwtp1 - Koh_wwtp3, Sil_port1, Sil_port3, Sil_wwtp2, Sil_wwtp3) was not determined directly but was back-calculated from organic content measurements performed according to the loss on ignition method (Heiri et al., 2001). The latter procedure is justified by very strong correlation between TOC and organic content found in the studied sediments ($R^2 = 0.95$, $n = 17$, $p < 0.0001$).

2.2. Fieldworks

Collection of the sediment samples was performed onboard research vessels Salme (in the Estonian part of the Gulf of Finland), Maria and SN-1303 (in the Russian part of the Gulf of Finland). CTD (conductivity-temperature-depth) profiles for temperature and salinity data were measured by SBE-32 (Seabird) and Idronaut (S.r.l.), on board Russian and Estonian vessels, respectively. Van Veen and Ekman-Birge type bottom samplers or a plastic container (only at the shallow beach areas), were used to sample the upper sediment layer (0–5 cm). In addition, deeper sediment layers (up to 15 cm, with 5 cm interval) were collected in the Narva Bay (station N) by GEMAX sediment corer.

Sediment samples were collected in areas next to ports, within probable sediment accumulation areas, along routes of heavy marine traffic, in the vicinity of wastewater treatment plants, near the shore (depth below 15 m), and along the coastal beaches (in the shallow water, depth 0.5–1 m) (Fig. 1). Temperature, salinity, and dissolved oxygen content were measured in the seawater from surface and near-bottom layers, where possible. Water samples for the analysis of nutrient concentrations (total nitrogen/phosphorus, nitrates/phosphates) and sediment samples for the analysis of sediment characteristics (e.g., granulometric composition) were collected at most sampling locations.

2.3. Laboratory analysis

Hazardous substances from the collected sediment samples were analysed in Germany and Russia in the laboratories accredited for the

Table 1
Information on chemical analyses of sediment samples in 2019–2020. The results for sediment analysis are reported on a dry-weight (DW) basis. Laboratories that conducted analyses: [a]– GBA Gesellschaft für Bioanalytik mbH, [b]– VSEGEI Russian Geological Research Institute, [c]– ECOLAB LLC Laboratory, [d] – Eurofins Umwelt Ost GmbH (Freiberg). Thresholds for good environmental status are taken from the following sources: [1]– HELCOM, 2018c, [2]– HELCOM, 2018d, [3]– HELCOM, 2018e, [4]– OSPAR, 1998.

Parameter	Lab	LOQ	Method	Threshold	Number of stations
Cd	GBA[a] VSEGEI[b] ECOLAB[c]	0.1 mg/kg 0.01 mg/kg 0.01 mg/kg	ICP-MS ICP-MS ICP-OES	2.3 mg/kg DW[1]	56
Pb	GBA[a] VSEGEI [b] ECOLAB[c]	1 mg/kg 1 mg/kg 1 mg/kg	ICP-MS ICP-MS ICP-OES	120 mg/kg DW[1]	56
Cu and Zn	GBA[a] VSEGEI[b] ECOLAB[c]	1 mg/kg 1 mg/kg 1 mg/kg	ICP-MS ICP-MS ICP-OES	50 mg/kg DW (for Cu)[4]	56
TBT	GBA[a] ECOLAB[c]	1 μg/kg 10 μg/kg	GC GC–MS	1.6 μg/kg DW (5% TOC)[2]	35
ANT	GBA[a] ECOLAB[c]	2 μg/kg 1.2 μg/kg	GC–MS HPLC-FD	24 μg/kg DW (5% TOC)[3]	52
TOC	Eurofins[d] ECOLAB[c]	 0.1 W % DW	Dry combustion Wet oxidation		52

LOQ - limit of quantification, ICP-MS - inductively coupled plasma-mass spectrometry, ICP-OES - inductively coupled plasma-optical emission spectrometry, GC - gas-chromatography, GC–MS - gas-chromatography-mass-spectrometry, HPLC-FD - high-performance liquid chromatography with fluorescence detection.

used analytical methods (Table 1). Total metal concentrations were determined using inductively coupled plasma-mass spectrometry (ICP-MS) or inductively coupled plasma-optical emission spectrometry (ICP-OES), detection of the organotin compounds was conducted through gas-chromatography (GC), PAHs were determined using gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography with fluorescence detection (HPLC-FD), following HELCOM guidelines. The nutrient analyses were performed according to the recommendations made by USEPA, ISO, and DIN standards. Dissolved oxygen measurements were obtained from the water column profile data validated by potentiometric titration.

2.4. Data processing and visualization

Pearson's correlation coefficients with a significance level of 5% were applied to determine relationships between various parameters using software R (R Core Team, 2020). Prior to the correlation analysis, the HS concentration values less than the limit of quantification (<LOQ) were replaced by 50% of the LOQ in the datasets, i.e., we inferred that the measured value lay between zero and the LOQ. According to HELCOM recommendations, the normalization of ANT and TBT contents to the 5% TOC was done to enable the spatial comparison from various sediments.

High-temperature combustion processes are responsible for the emission of the PAHs with high molecular weight, mainly with four or more rings (Wang et al., 1999; Stogiannidis and Laane, 2015). In the study area, the sum of 4–6-ringed PAHs typical for combustion ($\Sigma PAH_{combust}$) was calculated as the sum of FLU, PYR, BAA, CHR, BBF, BKF, BAP, ICDP, DBAHA, BGHIP (e.g. acc. Barrick and Prahl, 1987). The total concentration of potentially mutagenic and genotoxic PAHs (ΣPAH_{tox}) was calculated as the sum of BAA, BBF, BKF, BAP, CHR, ICDP, DBAHA (acc. to WHO, 1989).

The potential effect of the studied PAH compounds and trace metals was assessed according to sediment quality guideline (SQG) obtained from Long et al. (1995). The measured HS concentrations were compared to guideline values of lower reference value - ERL ("effect range-low") and upper reference value - ERM ("effects range-medium") associated respectively with low to high probable biological effect. In order to determine the potential degree of the risk the heavy metals might pose to the environment, we applied the following environmental indicators:

- Contamination factor (CF)

$$CF = \text{Concentration}_{\text{metal}} / \text{Concentration}_{\text{background}}$$

The average concentrations of trace metals until the 1930s in the Narva Bay (corresponds to the Pb-210, Ra-226, Cs-137, Am-241 dated sediment profiles at the depth of 18–32 cm, obtained using GEMAX corer in 2014 from the Narva Bay) were defined as background values according to the results of the geological survey of the eastern Gulf of Finland (SEDGOF, 2016). Contamination factor has four categories in sediments: <1: low contamination, 1–3: moderate contamination, 3–6: considerable contamination, >6: very high contamination (Hakanson, 1980; Nour and Nouh, 2020).

- Potential contamination index (CP)

$$CP = \text{Concentration}_{max.\text{content}} / \text{Concentration}_{\text{background}}$$

Potential contamination index has three categories in sediments: <1: low contamination, 1–3: moderate contamination, >3: severe or very severe contamination (Hakanson, 1980; Nour and Nouh, 2020).

- Potential ecological risk index (PERI)

$$PERI = \sum_i^n (Trf * CF)$$

where *Trf* is a toxic response factor, for Cd, Pb, Cu and Zn are 30, 5, 5 and 1, respectively (Hakanson, 1980), CF is the contamination factor of the single heavy metal defined in the sample. The degree of ecological risk can be categorized as follows: <40: low risk, 40–80: moderate risk, 80–160: considerable risk, 160–320: high risk and ≥320: very high risk (Hakanson, 1980; Qing et al., 2015; Nour and Nouh, 2020).

The degradation rate of organotin compounds depends on various physical, chemical, and biological factors; hence, TBT retention in the marine sediments might last from months to tens of years (Takeuchi et al., 2004; Viglino et al., 2004; Jokšas et al., 2019). The differentiation between probable sources of TBT contamination attributed to recent and continuous input or long-standing incident was done by calculating indices based on the ratio of TBT and its less toxic degradation derivatives DBT and MBT (Michel et al., 2001). A higher value of TBT to DBT ratio (R_{TBT}) relates to lower degradation of TBT, indicating fresh contamination (Üveges et al., 2007), as well as the ratio of MBT + DBT to TBT (BT degradation index-BDI) below 1.0 (Díez et al., 2002).

2.5. Hydrodynamic model

Numerical modelling was applied to show the prevalent pathways and distribution of the HS considering atmospheric forcing and topographic features of the aquatic environment in the eastern Gulf of Finland. Circulation simulation with a high-resolution nested setup of the GETM (General Estuarine Ocean Model, Burchard and Bolding, 2002) for the entire gulf was performed for the period 01.01.2018 to 31.12.2019 using three classes of dye tracers with constant settling velocities to highlight possible accumulation zones within the gulf. The simulation period corresponds to the water residence time of 2 years in the Gulf of Finland (Alenius et al., 1998; Liblik and Lips, 2017).

GETM is a free-surface, hydrostatic, primitive equation ocean model with a k-epsilon vertical turbulence model embedded via GOTM (General Ocean Turbulence Model; Umlauf and Burchard, 2005). Tracers in the model are implemented via FABM (The Framework for Aquatic Biogeochemical Models; Brüggeman and Bolding, 2014) model. More specifically, IOW SPM (suspended particulate matter model by Leibniz Institute of Baltic Research, Gräwe and Wolff, 2010; Osinski and Radtke, 2020) has been used.

The horizontal grid spacing of the nested model is 250 m and 60 vertically adaptive coordinates (Hofmeister et al., 2010) are used. Gräwe et al. (2015) have shown that the latter produce less artificial numerical mixing in the simulations. Lateral boundary conditions for the temperature, salinity, sea surface height and current velocities are taken from a 1 km GETM model setup for the whole Baltic Sea with hourly resolution and interpolated to the horizontal grid of the nested model. More details about the coarse resolution model setup are given in Zhurbas et al. (2018) and Liblik et al. (2020).

Atmospheric forcing was taken from the Estonian version of the HIRLAM (High Resolution Limited Area) model maintained operationally by the Estonian Weather Service and giving forecasts with hourly resolution ahead of 54 h (Männik and Merilain, 2007). River runoff data was taken from a dataset prepared for the BMIP (Baltic Model Intercomparison Project) by Väli et al. (2019) consisting of hydrological hindcast and forecast data from the E-HYPE model (Lindström et al., 2010).

Idealistic scenarios with dye tracers with constant settling velocity were simulated. As we are interested in studying the universal pathways of riverine origin and not a particular HS class, the concentrations of the tracers were set to 1 for all the Gulf of Finland rivers. In this first attempt to model the pathways of HS, we assumed constant HS concentrations in rivers. Thus, the load is proportional to the freshwater discharge with its seasonal variability. Values for the settling velocity were 10 cm/day (light particles), 50 cm/day (medium particles) and 100 cm/day (heavy particles), which correspond roughly to the particle sizes less than 0.015 mm, i.e. fine silt for all tracers. Such small settling velocity allows us to better estimate the advection and sedimentation of riverine origin material, as with larger values, the sedimentation would occur in the close vicinity of the river mouths. The resuspension of the particles in the idealistic simulations is turned off to indicate the early accumulation areas after release.

3. Results

3.1. Environmental background data

Salinity measured at the sampling locations varied between around 0.2 at the shallow areas (approx. 1 m depth) in the vicinity of the Neva and Narva river mouths, and 8.0 g/kg in the near bottom layer at the deepest station LL3A (68 m) in the eastern Gulf of Finland. The oxygen and PO_4 content in the near-bottom layer revealed a strong negative correlation ($r = -0.72$, $n = 29$). The lowest oxygen concentrations (2 mg/l), which might be considered as hypoxia (Ærtebjerg et al., 2003), along with the high PO_4 content (361 μg/l), were marked at station LL3, however the highest PO_4 value was registered at the outer part of the Neva Estuary (station 2UGMS, 443 μg/l). Simultaneously oxygen depleted bottom conditions (3 mg/l) were characterized by the elevated level of PO_4 (340 μg/l) at deep offshore station 17F. One exceptionally high PO_4 value (339 μg/l) was measured at a shallow depth in Aseri during summer 2020.

The largest content of TOC was registered from deep sediment accumulation area near the northern coast of the eastern Gulf of Finland (stations LL3A, XV1, and 20F; 10, 8.3, and 7% of sediment DW, respectively). The TOC content below 0.1% was estimated from ten nearshore stations in the Estonian territorial waters, while at most stations the TOC values in sediment samples varied in the range of 0.1–5% (Table S3 in the supplemental data).

3.2. Concentrations of PAHs

The sum of PAH concentrations in the sediment samples in the studied Gulf of Finland area ranged between 13 and 7380 μg/kg sediment DW (Fig. 2, Table S1). At several stations in the western part of the study area, the values were below the LOQ. In the eastern part of the study area, the polyaromatic compounds were in all stations at levels above the LOQ (Table S1). The highest concentration was determined in ports of Muuga Harbour and Lomonosov (7380 and 4030 μg/kg, respectively), and near the former fuel oil storage in Aseri (2290 μg/kg, Fig. 2). Considerably lower concentrations were observed at the beach area near the port of Primorsk (971 μg/kg) and along the northern coast of the Gulf of Finland (station XV1, 933 μg/kg). The highest NAP content (1200 μg/kg) was detected near the probable point source of PAH contamination at Aseri, following more than 10-times lower value at the Port of Sillamäe (station Sil_port1; 99 μg/kg). The sample from hot-spot of PAH pollution – Muuga Harbour also contained the highest amount of FLU, PYR, BAA, BAP, CHR, ICDP, DBAHA, FLE, FLU, PA and PYR; while at other hot-spot - Port of Lomonosov sediments had the highest content of BBF, BKF and BGHIP.

When normalized for TOC%, sediments revealed notably high pollution by conditionally cancerogenic PAH, anthracene, in the port areas (Fig. 3). The highest value was found at Port of Lomonosov (2256 μg/kg), almost two times lower concentration registered at Muuga Harbour (1222 μg/kg), following by the station along the ship traffic route in Luga Bay (station 6 L) and the wastewater treatment plant (WWTP) Petergof (660 and 620 μg/kg, respectively; Table S3). TOC-normalized ANT sediment concentrations at those locations exceeded the suggested threshold for good environmental status (GES) more than 90, 50, and 25 times, respectively (Fig. 3). Values fivefold and higher than the GES threshold were estimated for the Ports of Primorsk and Kunda (325 and 125 μg/kg, respectively), at the shallow station at the Narva river mouth (station N_J, 195 μg/kg) and in the Luga Bay (stations GF5 and 18 L; 146 and 123 μg/kg, respectively). To a lesser degree, sediments from the shallow stations near dismantled oil storage in Aseri and next to the Sillamäe WWTP (110 and 94 μg/kg) contained anthracene above the threshold as well.

$SPAH_{combust}$ were present with concentrations from 9 μg/kg (at the Luga Bay) to 5900 μg/kg (in the Muuga Harbour) with an overall average concentration of 429 μg/kg (Table S2). The highest mean concentration was observed around the port areas (1599 μg/kg). The shares of these PAHs represented from 35% (at Aseri) to 100% (stations in the Neva Estuary) of the total PAHs concentrations with an average value of 78%. The proportion of $SPAH_{combust}$ was considerably higher in the Neva Estuary than in the other parts of the study area.

The $SPAH_{tox}$ ranged from 9 μg/kg to 3010 μg/kg with an average concentration of 298 μg/kg (Table S3). The share of these compounds averaged 58% of the sum of all PAHs and ranged from 20% to 85%, whereas in the Neva Estuary, the proportion of $SPAH_{tox}$ was considerably higher than in other parts of the study area as well.

The sum of low- and high-molecular weight PAHs, as well as the sum of all PAHs, were between ERL and ERM SQG values at the port areas of Muuga and Lomonosov (Table S1). At the shallow Aseri station, only low- molecular weight PAHs exceeded lower reference value ERL. From all compounds, only ANT was measured above the upper reference value ERM (1100 μg/kg) at the Port of Lomonosov. However, concentrations of single PAH compounds were found between ERL-ERM values at XVI and B_Izhora for DBAHA, and Luga Bay stations (18 L and GF5) for ANT.

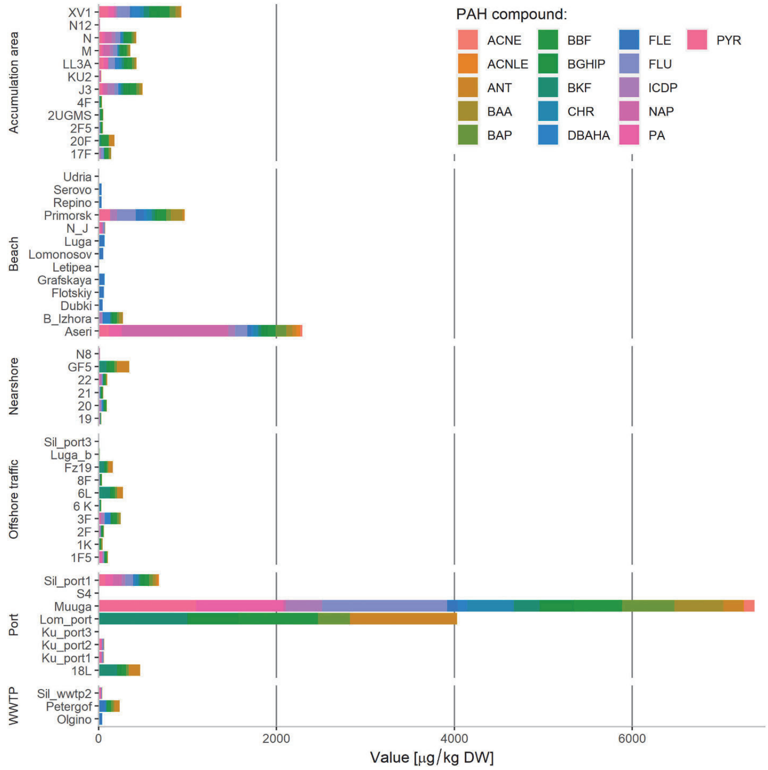

Fig. 2. Concentrations of PAH compounds from the sediment samples across the categorized locations in the study area.

3.3. Concentrations of trace metals

Among trace metals analysed from sediment samples, Cd concentrations were occasionally approaching and exceeding the threshold value for GES (Fig. 4, Fig. S1, Table S3). Cd polluted areas were mainly located in the central and northern parts of the Neva Estuary, while samples from the western parts of the study area had much lower concentrations.

In the coastal areas of the Neva Estuary, the Cd content in sediment samples was found previously to be unprecedentedly high (>20 mg/kg) mainly along the nearshore stations at the northern coast (Fig. 4). According to the recent survey, in most locations Cd did not exceed the GES threshold, except stations 20 (2.49 mg/kg) and 9F (3.04 mg/kg) in the inner and outer parts of the Neva Estuary (Table S3). Cd concentrations below 1 mg/kg were observed along the southern coast of the western part of the study area, while slightly elevated values were observed offshore in 2005–2018.

The highest Cu content (89 mg/kg) was registered at the Port of Lomonosov in 2019. The elevated Cu concentrations (50–55 mg/kg) were observed in 2019 and earlier in 2014 in the central part of the Neva Estuary near the monitoring station 17F (Fig. 5, Fig. S1, Table S3). The concentration of trace metal exceeded 50 mg/kg at station 20 in the inner Neva Estuary as well. The values were mostly below 20 mg/kg along the southern coast in the western part of the study area but higher offshore both in earlier and recent years.

The concentration of Pb exceeded 50 mg/kg (57–67 mg/kg) in the central part of the Neva Estuary both, in the previous period and according to the recent observations (Fig. 6, Fig. S1, Table S3). The latest data collected along the Estonian coast indicates that the content of Pb in the upper sediments has not surpassed the value of 25 mg/kg. Meanwhile, slightly higher values (>25 mg/kg) were found offshore; however, according to historical and recent data, Pb content never exceeded the HELCOM GES threshold in the study region.

Correlation analysis of studied variables shows a statistically significant correspondence between Cd, Cu, Zn and Pb concentrations ($r > 0.65$, $p < 0.0001$). The co-occurrence of Pb concentrations higher than 30 mg/kg and total organotin compounds predominantly higher than 50 μg/kg is also reflected in the strong linear correlation between these contaminants in the sediment samples ($r = 0.84$, $n = 26$; Fig. 7b). Pb was negatively associated with oxygen content near the bottom ($r = -0.68$, $n = 51$; Fig. 7a), though positively correlated with sediment fine fraction content ($r = 0.67$, $p < 0.0001$, $n = 33$). The fraction content <63 μm correlated less strongly with Cd, Cu, and Zn ($r = 0.51$–0.58, all $p < 0.01$).

The threshold effect level (TEL), a minimal effects range below which adverse effects only rarely occurred, for Cu (18.7 mg/kg) according to the SQGs for US Florida coastal waters (Macdonald et al., 1996), might be related to the preindustrial contamination level in the study area (19.3 mg/kg; Table 2). Whereas ERL value, lower limit of the SQGs established in the US (Long et al., 1995), for Cd (1.2 mg/kg) is significantly lower than the HELCOM threshold value set for the Baltic Sea (2.3 mg/kg; Table 1).

The average concentration for Cd, Cu and maximum values for Zn, Pb in the eastern Gulf of Finland, along with maximum levels of these trace metals registered in the Neva Estuary, exceeded the ERL level (Table 2). At Muuga Harbour Zn, Cu and maximum level for Cu registered in the Luga Bay were above the ERL as well. The maximum Zn content in the Neva Estuary and Cu from Port of Lomonosov exceeded more than two times the ERL criteria for marine sediments.

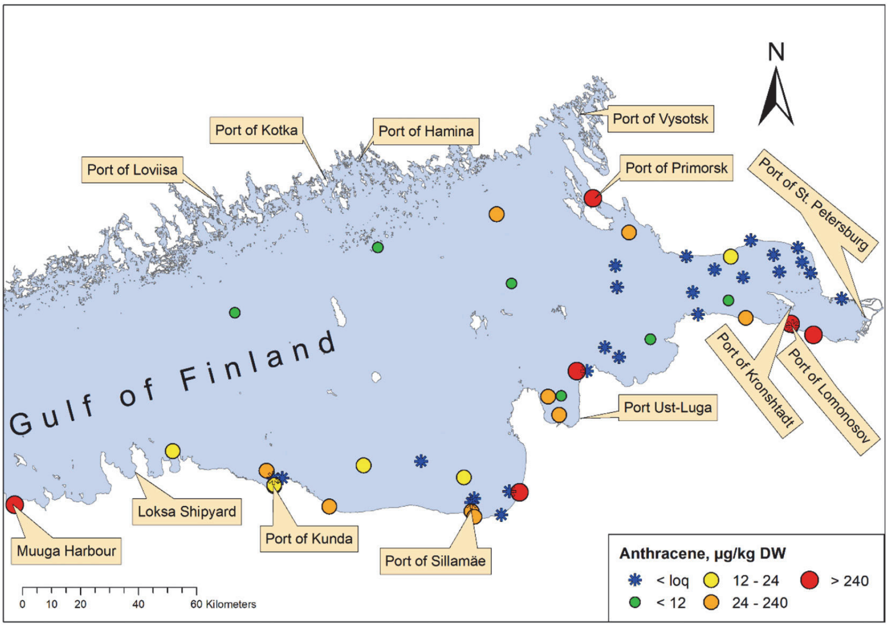

Fig. 3. Distribution of anthracene in sediments according to the data collected during the survey period 2019–2020. ANT concentration normalized for TOC content. Yellow spheres indicate the values between a half of the GES threshold and the GES threshold (24 μg/kg DW), orange spheres the values ranging from 1 to 10 times of the threshold, and red spheres the values exceeding the threshold more than 10 times. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

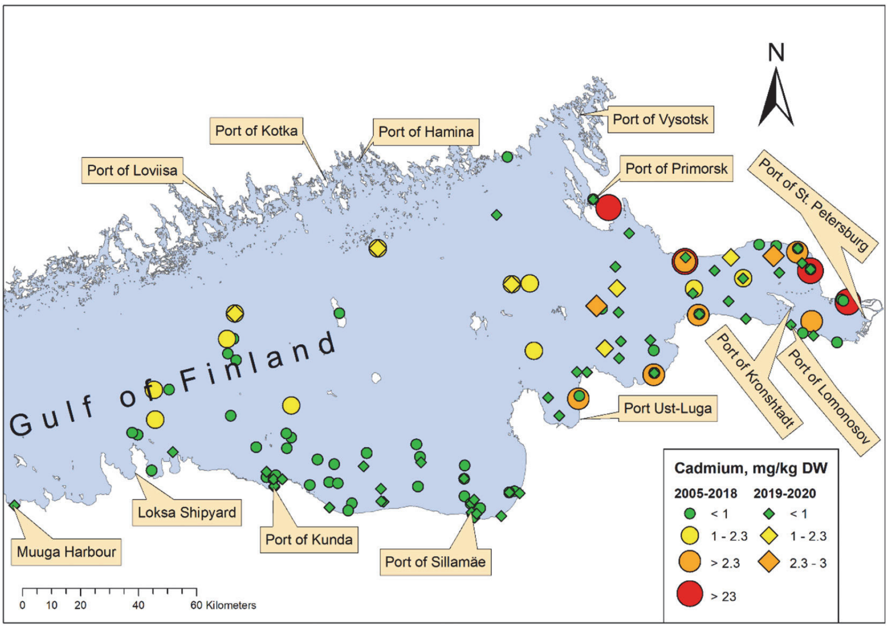

Fig. 4. Distribution of Cd in sediments according to the historical data (spheres) and data collected during the present survey (diamonds). Yellow spheres/diamonds indicate the values between 1 mg/kg and the HELCOM GES threshold (2.3 mg/kg DW), orange spheres/diamonds the values ranging from 1 to 10 times the threshold, red spheres the values exceeding the threshold more than 10 times. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

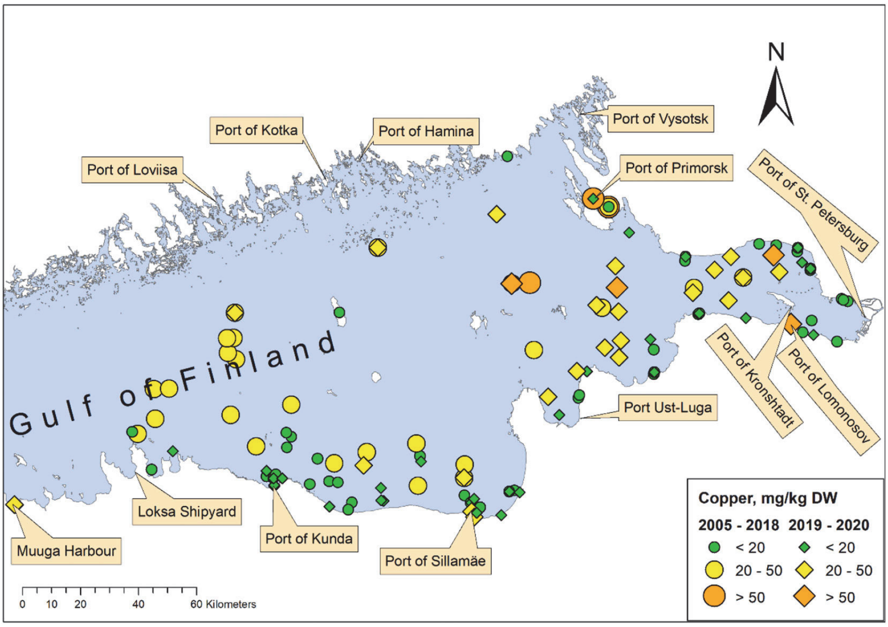

Fig. 5. Distribution of Cu in sediments according to the historical data (spheres) and data collected during the present survey (diamonds).

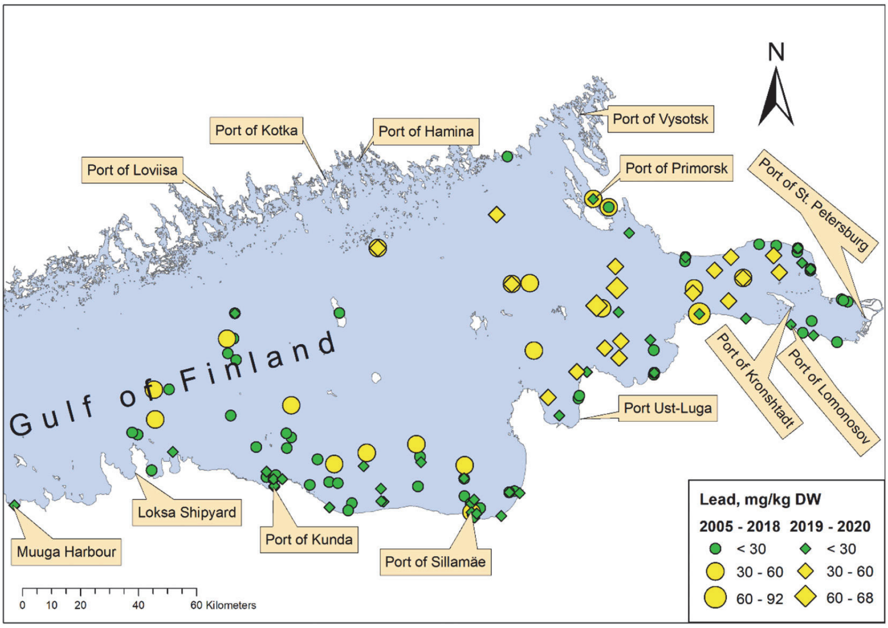

Fig. 6. Distribution of Pb in sediments according to the historical data (spheres) and data collected during the present survey (diamonds).

3.4. Concentrations of organotin compounds

The highest content of organotin compounds in the study area (>50 μg/kg) was found in the sediment samples across the Neva Estuary (Fig. 8). BTs mainly in the form of TBT and its degradation products, constituted more than 70% of organotins in all sediment samples where organotin compounds were above the LOQ. The concentrations of TBT, OTC or BTs had no statistically significant correlation with TOC content in the bottom sediment (p-value >0.05). However, weak correlation was observed between the fine sediment fraction content and TBT, OTC,

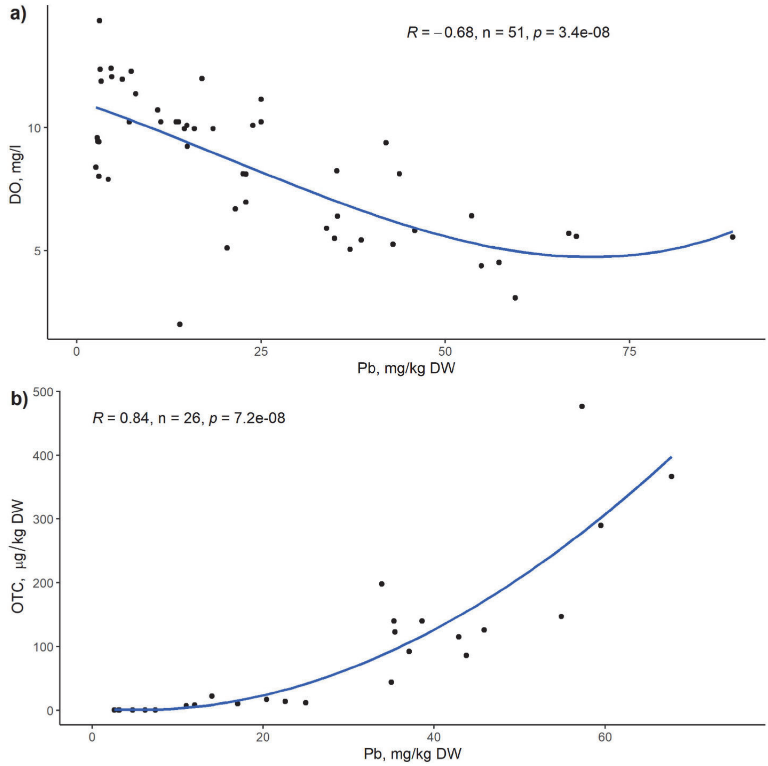

Fig. 7. Correlation between Pb in sediment and dissolved oxygen content (DO) in the near-bottom water layer (a) and concentration of organotins (OTC; b) the trendline present polynomial interpolation of the regression.

MBT ($r = 0.48$–0.51, all $p < 0.05$).

The positive correlation between TBT degradation products MBT and DBT at the sampled locations ($r > 0.8$, $p < 0.0001$) has been found, while no statistically significant correlation value was obtained between content of TBT and its metabolite DBT ($r < 0.4$, $p > 0.05$). At stations 1F5 and 22, the BDI index is the highest and from butyltins prevails degradation product DBT (content in sediment four and two times higher than TBT, respectively; Table S4).

In most sampled stations, TBT content prevails over other BTs and Rtbt index is >1. The Rtbt is negatively correlated with the BDI index ($r = -0.6$, $p < 0.01$).

At stations 19, 1 K and Luga Bay BDI <1.5 as TBT dominated among other butyltins, while concentrations of its degradation products (only DBT or both DBT and MBT) were under quantification limit of the analytical method used (Table S4).

Samples collected from the northern coast of the Neva Estuary (stations 19, 21, 22) and Luga Bay had low organic carbon content (less than 1% of sediment dry weight), and after normalization procedure, measured concentrations of TBT became >100 µg/kg (Table S4). At the same time, at stations from the western parts of the study area (Muuga, M, N, J3), the TBT content below 10 µg/kg after normalization to the 5% TOC exceeded 11 µg/kg. Consequently, at all surveyed stations, content of TBT was either below LOQ or exceeded the GES threshold value (Fig. 9).

3.5. Simulated accumulation areas

The model simulations indicate that HS could reach the highest accumulation rate in the sediments in the middle of the Neva River Estuary (Russia), small inlets of Finnish coastal areas, and shallow accumulation areas in the Estonian part of Narva Bay (Fig. 10). The extent and the pattern of the simulated sedimentation area are dependent on the settling velocity i.e., the particle size. The largest extent of the sedimentation area is obtained for the medium-sized particles (Fig. 10b), while it is the lowest for heavy particles, which settle in the closest vicinity of the river mouths i.e., the release locations. Only a fraction of the light particles has settled in the deep areas, but in general, all the sedimentation patterns are similar – initially released from the rivers, the sediments reach the bottom relatively quickly in the shallow coastal areas and the amounts are approximately 100 times smaller in the deep areas. Comparison of the integrated amounts in the water column and sediment loads with the cumulative loads during the simulation (not shown) indicates that approximately 60% of the light material has deposited into sediments during 2 years, while for the medium and heavy particles, the corresponding ratios are 90 and 98%, respectively.

4. Discussion

Trilateral collection of the HS data in countries surrounding the subregion of the eastern part of the Gulf of Finland revealed high contamination with several hazardous substances.

4.1. PAHs

The level of total hydrocarbons is monitored on a regular basis in the Russian part of the Gulf of Finland as contamination by them has been observed in all parts of the Neva Estuary (Mannio et al., 2016).

Table 2
The mean (with maximum, where available) concentrations of trace metals in the study area and several alternative regions. The preindustrial levels applied as background values and criteria from the sediment quality guidelines included TEL, ERL, ERM.

Location	Cd	Pb	Cu	Zn	Reference
Eastern Gulf of Finland	1.26 (1.86)	34.86 (59.5)	38.16 (51.7)	143.5 (210)	Present work
Muuga Harbour	0.2	12	42	164	Present work
Port of Kunda	<0.1	2.8	11	24	Present work
Port of Sillamäe	0.28	23	29	74	Present work
Narva Bay	0.2 (0.45)	5.89 (17)	9.98 (27)	39.11 (95)	Present work
Luga Bay	0.35 (0.8)	22.28 (35)	19.42 (42)	79.65 (138)	Present work
Neva Inner Estuary	0.66 (2.49)	31.81 (57.3)	25.08 (54.5)	94.37 (174)	Present work
Neva Outer Estuary	0.86 (3.04)	36.45 (67.8)	27.79 (50.4)	130.3 (305)	Present work
Port of Lomonosov	0.45	29	89	99	Present work
Naples harbour (Italy)	0.9	123	131	303	Adamo et al. (2005)
Mediterranean coast (France)	1.77	206.5	14.82	269.2	Fernex et al. (2001)
Res Sea coast (Egypt)	1.38	30.4	23.38	48.19	Nour and Nouh (2020)
Norwegian coast	0.6	7.5	12.2	31.3	Aslam et al. (2020)
Preindustrial level (Narva Bay)	0.17	22.2	19.3	82.6	SEDGOF (2016)
TEL	0.68	30.2	18.7	124	Macdonald et al. (1996)
ERL	1.2	46.7	34	150	Long et al. (1995)
ERM	9.6	218	270	410	Long et al. (1995)

Nevertheless, information on the most environmentally harmful hydrocarbons - PAHs is limited. The historical data on anthracene in sediments is available from a few sampling locations in the eastern Gulf of Finland, comprising the Estonian and Russian territorial waters. The concentrations previously measured were below LOQ. Among the PAHs, the phenanthrenes and fluorenes were the only publicly reported substances by far (Berezina et al., 2017), which belong to the low molecular weight PAHs along with naphthalene.

The naphthalenes are abundant in diesel (typically >80% of total PAHs) and gasoline fuels (>90%) (Stogiannidis and Laane, 2015). In the Aseri sediment sample, this acutely toxic compound was the most abundant among the PAHs (1200 μg/kg, constituted >50% of total PAHs), while at other sampling locations, the concentrations were more than 10 times lower. The high concentrations in Aseri might be related to the point-source pollution from the abandoned industrial oil storage reservoir, recently undergone profound remediation process. The elevated phosphate concentrations in the near-bottom water layer indicate either high external or internal nutrient loads. Episodic oxygen depletion may occur in the coastal zone of the Baltic Sea (Conley et al., 2011), especially in the presence of a large amount of decaying algal biomass (Norkko and Bonsdorff, 1996) which was also observed at Aseri. As shown earlier by experimental studies (e.g., Bauer and Capone, 1985) the depletion of oxygen may contribute to the conservation of PAH contaminants (e.g., naphthalene), otherwise degraded during biological processes. Further systematic observations are needed to confirm the phenomenon as such low oxygen concentrations were hardly observed before in the southern coast of the Gulf of Finland (e.g., Conley et al., 2011). However, the recurring anoxia events at the deep sediment accumulation areas are expected to cause the release of phosphates from bottom sediments. It could be the case at offshore stations LL3A and 17F, where the elevated concentration of phosphates and depleted oxygen conditions were registered simultaneously.

The levels of PAHs, collected in the surface sediments of the Gulf of Finland in 2002, were in the range of 600–850 μg/kg DW for the sum of 13 compounds (Pikkarainen, 2004). In our study, the sum of 16 compounds was the highest in the port areas exceeding the earlier results remarkably. The estuarine sediments are known to be the most significant sinks for PAHs in the aquatic environment (Means, 1998; de Boer et al., 2001), while in the Neva Estuary, the maximum level of

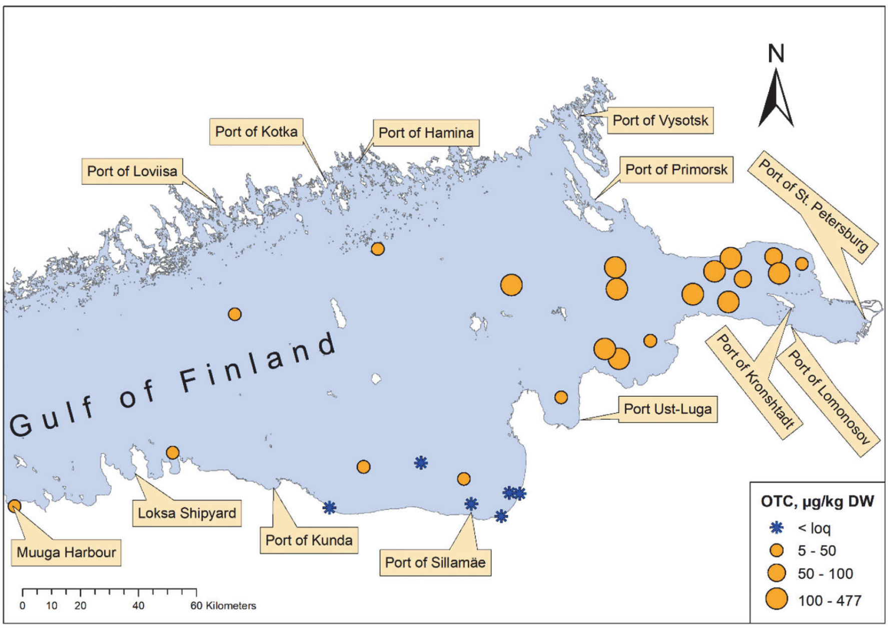

Fig. 8. Distribution of OTCs in the study area, size of the spheres indicates the total concentration of the organotin compounds found in the sediments.

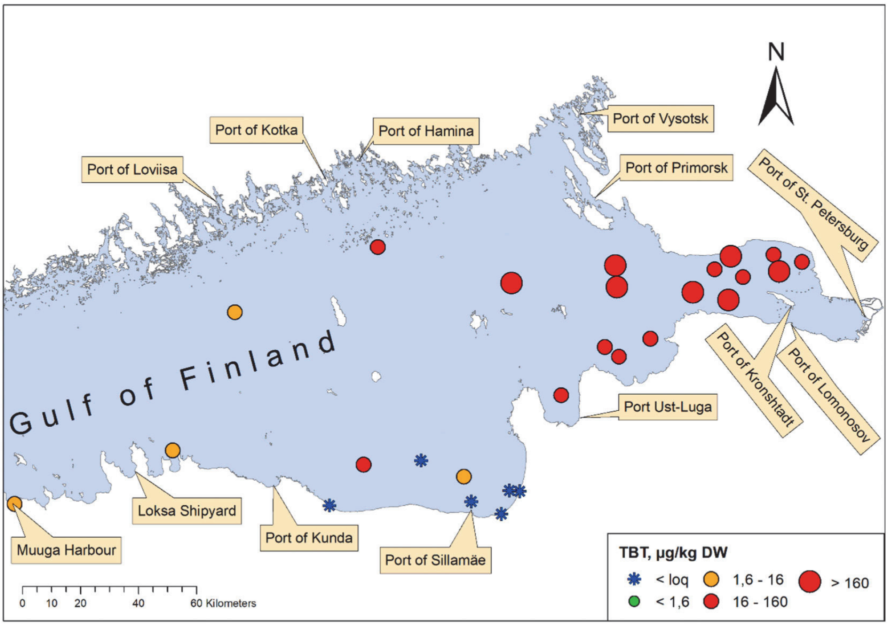

Fig. 9. Distribution of TBT (TOC-normalized) in sediments according to the data collected during survey period 2019–2020. Orange spheres indicate the values ranging from 1 to 10 times the HELCOM GES threshold (1.6 µg/kg DW), red spheres the values ranging from 10 to 100 times the threshold, and large red spheres the values exceeding the threshold more than 100 times. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contamination by PAHs (275 µg/kg) is much lower than in the sediments at Muuga Harbour and Port of Lomonosov (7380–4030 µg/kg). Thus, concentrations in these ports are similar to the highly industrialized port areas of the Southern Baltic (Falandysz et al., 2006; Stakėnienė et al., 2016). The total concentrations of PAHs in the studied harbours are lower compared with Basque harbours and estuaries (Spain), where near industrial areas and dredged sediment disposal sites quantities often exceed 15,000 µg/kg (Legorburu et al., 2014), and lower as well as the levels registered in the Oslo harbour (Norway)-12,000–18,000 µg/kg (Cornelissen et al., 2008). However, the observed content of SPAH is higher compared with the harbours of Olbia in Italy (160–770 µg/kg; De Luca et al., 2005) and Eastern Alexandria in Egypt (389.6 µg/kg; El Nemr et al., 2013). Such significant differences in the content of PAHs apparently highlight not only the extent of the local anthropogenic loading but also the presence of specific bottom conditions that facilitate accumulation and persistence of the sediment contaminants.

Among all studied areas, only sediments in Port of Lomonosov, considering ERM reference level, qualified to be highly contaminated by ANT (acc. to SQG of unnormalized data in Long et al. (1995)), indicating significant potential of negative impact on biota. Though, at port in Muuga Harbour, eastern GoF accumulation area (XVI), inner Neva Estuary (B_Izhora) and Luga Bay stations (18 L and GF5) concentrations of individual PAH compounds lied between ERL and ERM values, which might be related to occasional adverse effects on marine organisms. Meanwhile, during the assessment of environmental contamination based on HELCOM quality threshold values, factors controlling the bioavailability of organic pollutants (e.g. TOC content) in the contaminated sediments are important to consider as well. The environmental impact becomes more evident in the study area if considering the occurrence of certain PAH indicator compounds.

The result of the last assessment of the environmental status in the Baltic Sea regarding the contamination by acutely toxic low molecular PAH anthracene, despite the lack of information in some basins, indicated that the GES threshold was achieved in the northern parts of the Baltic, where concentrations ranged between 10 and 20 µg/kg (HELCOM, 2018e). However, based on the results of the current study, ANT was detected at 52% of samples and at 29% exceeded the GES threshold. The level of pollution with this substance in the eastern Gulf of Finland, specifically around the technogenic areas, is comparable to the Southern Baltic Sea, where normalized values surpass the threshold manifold (Fig. 11). The spatial pattern of ANT distribution in the study area clearly differed from distributions of organotins and heavy metals. The significant amounts of ANT were found mainly nearshore, but not detected in the central part of the Neva Estuary, which might be related to the point-source pollution from the certain objects situated near the coast (e.g., oil storage at the ports or harbours).

A widespread approach to distinguish between sources of PAH contamination in sediments is to apply PAH ratios. From the ratios of PAH isomers with different stability, ratio PA/ANT between 30 and 10 shows a mixed source profile with possible petrogenic character of contamination, while ratio FLU/PYR under 1.0 might indicate the petrogenic products and above 1.0 – pyrogenic sources (e.g., Stogiannidis and Laane, 2015). The ANT/ANT+PA ratio less than 0.1 usually indicates the petroleum residues, while ratio > 0.1 - a dominance of combustion as the source of input at the deposition site (acc. to Budzinski et al., 1997). The ratio FLU/FLU+PYR allows to deduce the character of combustion behind the PAH in a sample, as a fingerprint of liquid fossil fuels (incl. Vehicle diesel and gasoline) found in ratios between 0.4 and 0.5, whereas ratios >0.5 are characteristic more to wood or coal combustion (e.g., Yunker et al., 2002).

According to applied PAH molecular indices, the centre of the Neva Estuary (stations 22 and 3F, Table S2) and shallow coastal station near the Sillamäe Port/WWTP (Sil_WWTP2) distinguish from all other locations in the study area. A mixed profile of PAH sources with a possible petrogenic character of contamination (PA/ANT ratio > 10) was found at stations 22 and 3F. In addition, at station 3F (according to FLU/PYR

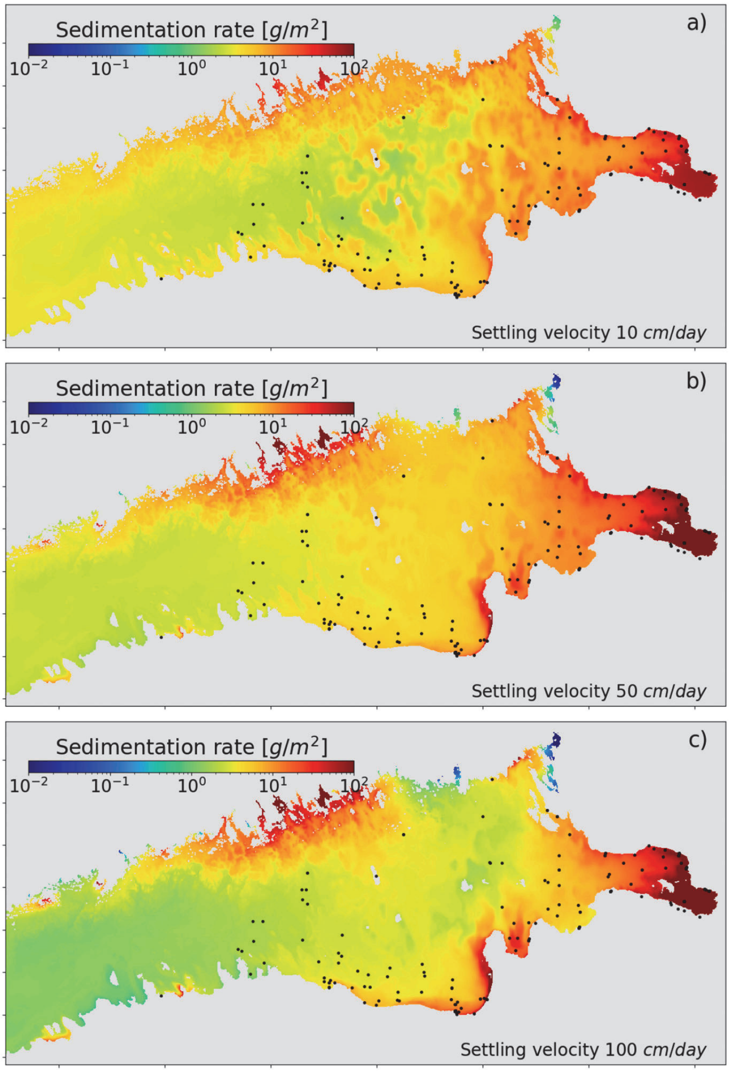

Fig. 10. Distribution of simulated sedimentation [g/m2] within two years of simulation with tracers released from the rivers. Values for the settling velocity in the simulations were 10 cm/day (a), 50 cm/day (b) and 100 cm/day (c). The locations of the available measurements of Cd in the upper sediment layer are shown with black dots.

and ANT/ANT+PA), station 22 (ANT/ANT+PA) and station Sil_WWTP2 (FLU/PYR), other calculated ratios similarly point to the same probable sources - petrogenic products. At stations 3F and Sil_WWTP2, evidence of a strong influence of combustion of liquid fuels (FLU / FLU + PYR <0.5) was found. However, corresponding to the index values, the rest of the port and sediment accumulation areas probably had pyrogenic sources of PAHs that originated from mostly solid fuels' combustion. The emissions from burning coal, oil-shale or wood are suggested sources for the input of those contaminants, while in the case of Muuga Harbour, the municipal solid waste incineration at the adjacent Iru Power Plant (Latõšov et al., 2018) might contribute to this observation as well.

The high molecular weight PAHs of pyrolytic origin are entering the waterbodies by atmospheric deposition or with contaminated soil (Budzinski et al., 1997; Morillo et al., 2008). The atmospheric deposition is the probable source of most of the PAHs at the offshore sediment accumulation areas sampled during the present study, while in the areas around the ports, adjacent to shipping routes, and near the beaches in recreational centres, input from contaminated soil cannot be excluded. The latter is confirmed by the fact that at most coastal stations around the Neva Estuary, the PAH contamination consisted of 100% low molecular weight compounds, which might be more toxic than high molecular PAHs (Kennish, 1997). PAHs degrade under aerobic conditions (Srivastava et al., 2017), while hypoxic conditions can support the longstanding stability of PAH compounds in the sediment matrix (Page et al., 1999; Short et al., 2007). Latter is the case for the deeper offshore and organic-rich areas of the Gulf of Finland, where hypoxic conditions establish at 50–70 m depth, specifically in the western and central Gulf of Finland during spring to autumn periods (Stoicescu et al., 2019). The coastal hypoxia, mainly induced by algae blooms, is reported from the Neva Estuary as a periodically occurring phenomenon as well (Berezina

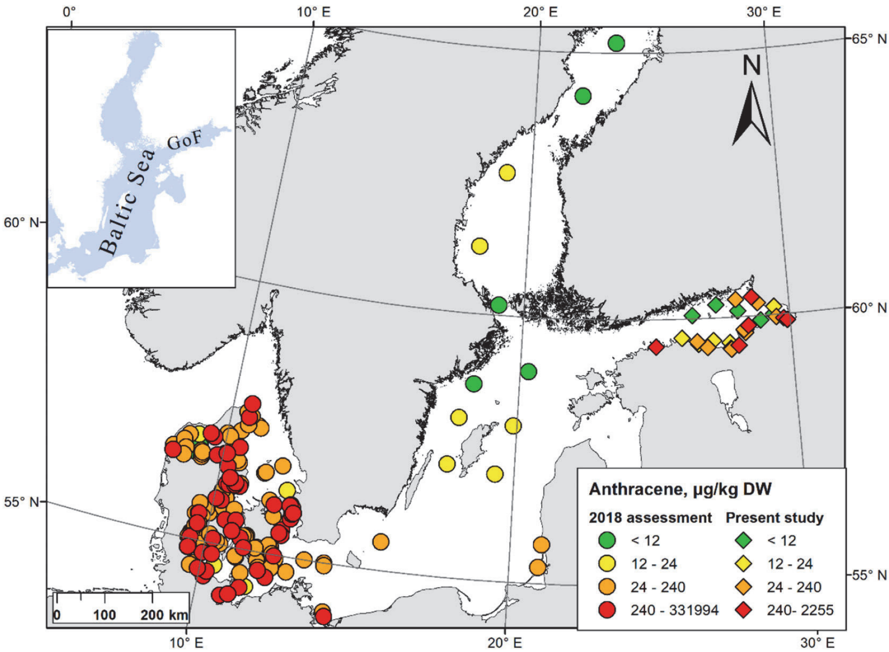

Fig. 11. Initial data from the HELCOM assessment on the anthracene (TOC - normalized). Green spheres/diamonds indicate the values below the HELCOM GES threshold (24 µg/kg DW), yellow spheres/diamonds - ranging between a half and the threshold, orange spheres/diamonds - ranging between 1 and 10 times of the threshold, red spheres/diamonds - exceeding the threshold more than 10 times. Data cover the period 1996–2016, and the assessment was conducted in November 2017 and published in June 2018: http://metadata.helcom.fi/geonetwork/srv/eng/catalog.search#/metadata/fb8ff223-9d6e-45cd-9357-82cf891f994e (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
An overview of the levels of PAHs, organotins and selected metals measured in different parts of the Baltic Sea, with focus on major harbour areas. NA = data not available.

Location	ΣPAH	TBT	ΣOrganotins	Pb	Cu	Zn	Cd	Reference
	µg/kg DW	µg/kg DW		mg/kg DW				
Bothnian Sea (station SR5)	2.6 (16 PAH)	<1	<2	0.8	0.5	3.3	<0.1	Berezina et al., 2019
Eastern Gulf of Finland	142.5–933.1 (16 PAH)	12–174	21.9–290	14–59.5	23–51.7	100–210	<0.1–1.9	Present study
Muuga Harbour	7380 (16 PAH)	2.3	8.2	12	42	164	0.2	Present study
Port of Kunda	60.2	<1	NA	2.8	11	24	<0.1	Present study
Port of Sillamäe	678	<1	<1	23	29	74	0.3	Present study
Narva Bay	<10–423	<1–5.6	<1–10.1	2.6–17	2.7–27	11–95	<0.1–0.45	Present study
Luga Bay	9.5–469	18	39	11.4–35	2.7–42	6.6–138	<0.1–0.8	Present study
Neva Bay	41–239	NA	NA	14.6	5.0	18.6	0.1	Present study
Neva estuary:								Present study
Inner	29.3–274.9	14–155	14–477	7.1–57.3	3.1–54.5	14.1–174	0.1–2.5	
Outer	30.6–159	17–240	17–367	7.1–67.8	4.3–50.4	21.5–305	0.2–3	
Port of Lomonosov	4030	NA	NA	29	89	99	0.45	Present study
Klaipeda strait	5456 (16 PAH)	130	NA	35.6	33.8	168	0.71	Galkus et al. (2012); Suzdalev and Gulbinskas (2014); Stakėnienė et al. (2016)
Port of Gdynia	2100 (16 PAH)	1200	NA	36	23	140	0.66	Falandysz et al. (2006)
Port of Gdansk	18,000 (16 PAH)	990	NA	61	34	270	2.1	Falandysz et al. (2006)
Kiel Harbour	22,100 (14 PAH)	2800	NA	118	62.3	185	NA	Baumard et al. (1999); Biselli et al. (2000); Nikulina et al. (2008)
Stockholm Harbour	22,900 (16 PAH)	390	NA	288	367	42	NA	Eklund (2017)

et al., 2017).

4.2. Metals

The Cu concentrations above 50 mg/kg in sediments are considered to present a probable risk to the environment (OSPAR, 1998). A markedly high level of sediment contamination by Cu (>50 mg/kg) at the Port of Lomonosov (Fig. S1, Table 3), in the central part and near the northern coast of inner part of the Neva Estuary might be related to the intensive ship traffic and marine construction, as after the ban of TBT contained antifouling paints, the potentially less harmful copper substitutes the toxic compound in the coating materials (Swedish Chemicals Agency, 1993). The loading of trace metals by the Neva River is also suggested as one of the main contamination pathways in the estuary (Panov et al., 2002). The regional background value for Cu at the Neva Bay coast is 18 mg/kg, while sediment samples are frequently reported to exhibit high levels of contamination by heavy metals, and it is probably related to expansion of areas with accumulating mud and dredging activities in the Neva Bay (Rybalko and Fedorova, 2008; Ryabchuk et al., 2017). Resuspension and transport of sediments are known to influence the distribution of heavy metals (e.g., Kalnejais et al., 2010). Thus, one can expect that Cu and other heavy metals are probably gradually transferred into the nearshore muddy accumulation areas on the sites located in the inner part of the Neva Estuary. Such distribution pattern qualitatively corresponds to our model simulations in the study area as well.

The most Cd contaminated sediments in the eastern Gulf of Finland region were historically found predominantly throughout the Neva Estuary, at the hot spot areas exceeding 20 mg/kg. In the upper layer of a sediment core sample collected at the outer part of the estuary in 2009, Cd concentration was registered above 2 mg/kg (Vallius, 2014). According to the present study, the mean concentration of Cd, within previously highly contaminated areas of the Neva Estuary, was below the threshold and slightly exceeded the values in the Bothnian and Åland Seas, which were recently assessed to be between 0.2 and 0.4 mg/kg (HELCOM, 2018c). However, samples from multiple stations in the Neva Estuary, both nearshore and offshore areas, contained Cd close or above the HELCOM threshold, indicating the continuous environmental issue despite progress due to the regional regulations. Similarly, no decreasing trend of Cd concentrations in the fish liver was observed in different Baltic Sea basins (Lehtonen et al., 2017). Based on available data, no decrease in concentrations can be assigned as extremely high Cd content determined in 2018 at the northern shore of the Neva Estuary (e.g., Primorsk, Flotsky, Dubki; shown by red spheres in Fig. 4.) from deeper nearshore areas, spatially not related to shallower beach areas sampled during 2019. The following surveys have to focus on deeper nearshore stations for the Cd trend assessment. In addition, at shallow beach areas continuous disturbance of the bottom surface by waves and absence of fine sediments for pollutant accumulation might hinder the objectivity of marine pollution assessment due to high dispersion and subsequently low contaminant concentration. In contrast, high content of the element was not found in the coastal zone of the western part of the study area. Although data from sediment cores indicate traces of historical contamination in this region, as at offshore accumulation areas, deeper sediment layer (5–25 cm, corresponded to the period 2000–1980) was classified as moderately contaminated with Cd, the concentration of which was up to 2 mg/kg (SEDGOF, 2016).

According to both available historical data and the recent sampling, the determined concentrations of Pb were below the suggested HELCOM threshold value (120 mg/kg). However, considering other relevant quality standards, at six stations, the trace metal content exceeded EQS (Environmental Quality Standards) for sediments, specifically, established national quality guidelines (in Estonia - 53.4 mg/kg) and the upper OSPAR provisional EAC (Environmental Assessment Concentration) level of 50 mg/kg. In comparison with different regions of the Baltic Sea, the mean concentration across the Neva Estuary was slightly above the values of the Bothnian and Åland Seas recently measured at levels between 20 and 30 mg/kg (HELCOM, 2018c). Though, according to the previous monitoring reports on the coastal zone of the Neva Bay, at a single station in 2011, the significant contamination (>120 mg/kg) was registered in the upper silty sediment layer (Ryabchuk et al., 2017). Therefore, sediments of the Neva Bay containing silty mud might be prioritized for further monitoring of Pb levels in this area to detect the temporal changes in the Pb concentration.

The Cd concentrations in the studied port areas were lower than found in the Mediterranean Sea and Norwegian coast (Table 2). The mean concentration of Cd for the eastern Gulf of Finland sediment accumulation area exceeded the levels of contamination in the Naples harbour and Norwegian coast, at the same time being more than seven times higher than the average background preindustrial level of 0.17 mg/kg. At all sampled locations, concentrations of Pb were below the values found in Naples and the French Mediterranean coast, though the levels in the Neva Estuary and deep eastern Gulf of Finland stations were above the Red Sea and Norwegian coasts. The concentration of Cu in most sampled areas was above the preindustrial level, up to five times in the Port of Lomonosov, but still below the values measured in the Naples harbour. The difference in the mineralogical content of the sediments, expected in different regions, to some extent, explains the variability in the trace metal distribution.

The metal distribution in the sediments is known to be associated with redox potential. For instance, oxidized sediments of the Åland and the Bothnian Seas were found to have lower concentrations of trace metals than sediments from anoxic Baltic Proper (Borg and Jonsson, 1996). The low oxygen conditions (<5 mg/l) were observed at multiple locations in the outer Neva Estuary and the deepest accumulation areas. In the easternmost part of the Gulf of Finland increased concentration of Pb coincided with decreased oxygen levels. A combined effect of widespread eutrophication, consequent oxygen depletion and contamination by toxic metals are suggested to characterize this area earlier (Panov et al., 2002) and might be confirmed by our study as well.

The low concentration of dissolved oxygen in the overlying bottom water, along with sedimentation of organic matter, increase the lability of Pb-sediment complexes within underlying sediment and may increase the bioavailability of Pb (Chakraborty et al., 2016). Consequently, under such conditions, the benthic communities are expected to have higher exposure to the toxic metals, though the specificity of combined effects of multiple stressors (acc. to Nikinmaa (2013)) must be considered before any conclusions can be drawn on consequences of such interaction.

The assessment of sediment contamination by heavy metals was based on environmental indicators. CF and CP presented ratios between determined concentrations in the sediment samples and preindustrial levels measured in the Narva Bay as background values (Table 4). According to these indices, eastern Gulf of Finland sediments are highly contaminated with Cd (CF > 7, CP > 10). Calculated CP indicate that both parts of the Neva Estuary and Luga Bay might potentially suffer from severe contamination by Cd, while the outer part of the Estuary might also be highly contaminated by Pb and Zn (CP > 3). Moreover, the high risk of contamination for offshore eastern Gulf of Finland area and the outer Neva Estuary is confirmed by the potential ecological risk index (160 ≤ PERI < 320). The samples from the inner part of the Neva Estuary and Port of Lomonosov revealed a considerable ecological risk of contamination by studied heavy metals (80 ≤ PERI < 160). Though, careful interpretation of these values needed, as hydrodynamically complex processes in the vicinity of the river mouths trigger changes in the bottom conditions (through erosion or resuspension of the upper sediments) followed by an uneven accumulation rate of contaminants (Hakanson, 1980). The latter fact adds uncertainty to the ecological risk calculations based on in situ surveys in such locations.

Co-occurrence of elevated levels of contamination by Pb and OTC might point to the heavy marine traffic and continuous atmospheric deposition from the neighbouring industrial areas as a possible source of

Table 4
The environmental pollution indicators based on the trace metal concentrations in the sediment samples from the study area. Colours in the table indicate the degree of pollution according to the risk categories: CF (contamination factor), red - very high contamination, yellow - considerable contamination; CP (potential contamination index), red - severe or very severe contamination; PERI (potential ecological risk index), red - high risk, yellow - considerable risk; E^i_r (ecological risk index).

Location	Indicator	Cd	Pb	Cu	Zn	PERI
Eastern Gulf of Finland	CF	7.41	1.57	1.98	1.74	241.83
	CP	10.94	2.68	2.68	2.54	
	E^i_r	222.35	7.85	9.89	1.74	
Neva Inner Estuary	CF	3.89	1.43	1.3	1.14	131.56
	CP	14.65	2.58	2.82	2.11	
	E^i_r	116.75	7.16	6.5	1.14	
Neva Outer Estuary	CF	5.04	1.64	1.44	1.58	168.22
	CP	17.88	3.05	2.61	3.69	
	E^i_r	151.23	8.21	7.2	1.58	
Luga Bay	CF	2.05	1	1.01	0.96	72.63
	CP	4.71	1.58	2.18	1.67	
	E^i_r	61.62	5.02	5.03	0.96	
Narva Bay	CF	1.19	0.27	0.52	0.47	40.12
	CP	2.65	0.77	1.4	1.15	
	E^i_r	35.74	1.33	2.58	0.47	
Muuga Harbour	CF	1.18	0.54	2.18	1.99	50.86
	E^i_r	35.29	2.7	10.88	1.99	
Port of Lomonosov	CF	2.65	1.31	4.61	1.2	110.2
	E^i_r	79.41	6.53	23.06	1.2	
Port of Kunda	CF	<1	0.13	0.57	0.29	<35
	E^i_r	<30	0.63	2.85	0.29	
Port of Sillamäe	CF	1.65	1.04	1.5	0.9	63
	E^i_r	49.41	5.18	7.51	0.9	

these pollutants. The diffuse sources of heavy metals in the western region of the study area are known to be oil shale fuelled power plants, chemical industries related to oil and cement production (Liiv and Kaasik, 2004; Raukas, 2010). However, atmospheric emissions of heavy metals tend to steadily decrease in the European Union, as reported, the decrease during 1990–2012 was 89% for Pb and 66% for Cd (Tista et al., 2014). It is possible to obtain information from different layers of the sediment core sample to reveal the rate of contamination over the past decades. Based on the dated measurements from the core within the accumulation area in the central Neva Estuary, Pb concentrations are known to slightly decrease from the 1990-s as well, though concentrations of Cd have not shown a sign of decrease comparable to that observed for Pb (Vallius, 2014). While in Estonian territorial waters of the eastern Gulf of Finland the level of toxic heavy metals in the upper layer of bottom sediments, according to the measurements from the core samples, has stabilized or even shows a declining trend (SEDGOF, 2016).

4.3. Organotins

Accumulation of many organic pollutants, including organotin compounds, is known to be promoted by the specific sediment characteristics. The level of organic carbon fraction in the sediment has been shown to be potentially related to organotin contamination (Filipkowska et al., 2011; Suzdalev et al., 2015). According to our study, even though in the inner part of the Neva Estuary at stations 21, 22 and 2F, the silty sediments were relatively low in TOC content (around 1%), the total concentration of organotin compounds still exceeded 90 μg/kg. The high organotin content in the samples irrelevant of organic carbon fraction content probably arose from strong local anthropogenic input. A similar pattern was observed in the Arkona Basin, Southern Baltic, and was attributed to the residual pollution from World War II (Abraham et al., 2017).

The previous study conducted in the eastern Gulf of Finland, reported total organotins in the Finnish coastal areas at concentrations of around 50 μg/kg (Hallikainen et al., 2008). Substantially higher concentrations, with a maximum of 477 μg/kg were observed in the coastal and central parts of the Neva Estuary during the present study. However, in the areas closest to the sources of fresh input of organotin pollutants, as Copenhagen Harbour or at the docks of the Baltic Shipyard in the Tallinn Bay, the sediment samples may locally contain a much higher amount of total organotin compounds, which exceeds tens of thousands of μg/kg (Roots and Roose, 2013; Berezina et al., 2019).

Data on organotin compounds found at stations 1F5 and 22 indicate relatively old contamination, as BDI index is the highest and among the butyltins, TBT degradation product DBT (content in sediment is four and two times higher respectively than TBT) prevails. According to Okoro et al. (2011), this might point to the sources where PVC stabilizer or polyurethane foams might be manufactured. However, the overall non-significant, low correlation between the TBT and its metabolite DBT content might rather indicate the effect of restricted TBT degradation progress in the area.

The maximum amount of TBT in the upper sediments, measured in the outer part of the Neva Estuary (240 μg/kg), is comparable to the quantities reported for Klaipeda strait, where concentrations were repeatedly measured above 100 μg/kg (Table 3; Jokšas et al., 2019) and approach the level of pollution in the Stockholm Harbour, where TBT concentration close to the shipping lane was 390 μg/kg (Eklund, 2017). Despite the significant contamination level by PAH compounds in the sediments collected on the shipping lanes in three port areas along the southern coast of the Gulf of Finland (Kunda, Sillamäe and Muuga), TBT concentration was only up to 2.3 μg/kg. Much higher concentrations have been registered in the Southern Baltic, e.g., up to 1200 μg/kg in the Port of Gdynia (Falandysz et al., 2006). Likewise, the level of TBT contamination in the study area can be assessed by comparison with other relevant SQGs. According to the classification, set by Waite et al. (1991) for the UK estuarine sites, only sediments at the Neva Estuary accumulation area (station 2UGMS) indicate the level of contamination between medium and high (200–300 μg/kg), meantime, multiple stations from the Neva Estuary belong to medium contaminated sites (200–300 μg/kg), while all other stations, where TBT was quantified, might be classified as lightly contaminated. Considering the SQGs applied in Australia (Simpson et al., 2013), TBT almost at all stations in the Neva Estuary, one deep accumulation site (station 17F) and Luga Bay exceed the lower trigger value of 22 μg/kg, and might be termed as a contaminant of potential concern. Moreover, according to Australian SQG, upper trigger value of 70 μg Sn/kg (or 170.8 μg/kg) is exceeded at 2UGMS, 17F, and therefore harmful impact of TBT on benthic communities might be expected there. However, considering SQGs developed for the different marine regions, the comparisons need to be interpreted with caution, as an evaluation of environmental risk associated with the exceedance of the threshold values for organic pollutants has to follow the approximations associated with contaminant bioavailability (e.g. TOC content, specific sediment mineralogy and etc.), which are expected to differ regionally. Given that fact, the results of comparisons with different SQGs, might confirm the evidence of pollution extent, but not fully reflect the specific scope of environmental issues relevant to the Baltic Sea basins.

In most places of the study area, the TBT content prevails on other BTs, which may show the fresh input of this organic pollutant to the aquatic environment. R_{tbt} is negatively correlated with BDI and that is consistent with an evidence that most recent TBT contamination also reflected in the highest ratio of TBT to DBT in the accumulation zones - in the middle of the Neva Estuary (stations 3F, 2F5 and 2UGMS), both along northern and southern coasts of the study area (stations XV1, LL3A and M, N, J3).

Samples collected from the northern coast of the Neva Estuary (stations 19, 21, 22) and Luga Bay had low content of organic carbon (less than 1% of sediment DW), hence after the normalization procedure, normalized concentrations were much higher than the measured TBT concentrations pointing to the most severely polluted areas in the eastern Gulf of Finland (>100 μg/kg TOC-normalized). Stations from the western parts of the study area (Muuga, M, N, J3) with the TBT content below 10 μg/kg after normalization to the 5% TOC, exceeded the HELCOM GES threshold almost ten times.

The actual TBT concentrations in the study area, following normalization to the TOC, increased substantially, indicating that from 35 stations, where organic pollutant was measured, at 21 the level of contamination exceeded the GES threshold, in the inner part of Neva Estuary (station 3F) surpassing more than 300 times the established quality criteria. A similar pattern was observed in the Danish straits, where only two stations from 138 met the quality criteria for sediments (HELCOM, 2018d). The distinctive diffusive sources of TBT input are evident for the Neva Estuary. The overall distribution pattern of this organic pollutant differs from distributions of PAHs and heavy metals, demonstrating the continuous environmental issue historically related to the intensive maritime traffic in the region.

4.4. Simulated accumulation areas

Results from the idealistic model simulations qualitatively correspond to the observed accumulation patterns in the study area. The largest pool in the hazardous substances is close to the largest river in the Baltic Sea – the Neva River. Similar results are seen in Fig. 4, where the Cd concentrations in the sediments are the largest in the easternmost part of the gulf - the Neva Bay and along the northern coast of the Neva Estuary. Sedimentation of the medium size particles shows amounts growing from 28°E to the east, which can be in accordance with the distribution of the studied heavy metals (Figs. 4–6). Obviously, the short-term accumulation occurs only in the shallow regions and if the particles reach deeper areas, then their settling on the seabed will last much longer, and before that, the particles can advect and travel in the water column.

Results indicate that even with a very small settling velocity (0.1 m/day), it is hard for the hazardous substances of riverine origin in the particulate form to escape the vicinity of the river and accumulate in the deepest areas of the gulf.

5. Conclusions

The current study points out that some persistent organic pollutants in accumulation areas and around centres of the maritime activities in the eastern Gulf of Finland can be slightly below or manifold exceed the suggested GES thresholds for the Baltic Sea.

The significant contamination by organotin compounds was detected in sediments of the Neva Estuary. The organotins were found at lower levels in multiple deep areas of the eastern Gulf of Finland as well. According to our assessment, there is evidence of continuous fresh input along the ship traffic routes and probably restricted TBT degradation at deep sediment accumulation areas. The content of TBT exceeded the GES threshold value at 60% of the sampled stations. The overall distribution pattern of TBT differs from distributions of PAHs and heavy metals, likely depending on the presence of specific diffusive sources related to the maritime activities. The highest contamination by PAHs was found near the port areas, where compounds released during incomplete fuel incineration processes are likely prevailing. The situation with PAH contamination indicator in sediments – anthracene, raise the overall concern as it was detected at 52% of samples and at 29% exceeded the GES threshold, reaching extremely high values near the port areas. The spatial pattern of ANT distribution in the study area clearly differed from distributions of organotins and heavy metals and might be related to the point-source pollution from the certain objects situated near the coast (e.g., oil storage reservoirs at the ports or harbours).

The potentially toxic heavy metals Cd and Pb are still traceable in significant amounts in the Neva Estuary and deep offshore areas of eastern Gulf of Finland while it is not a concern along the northern Estonian coast. The Pb content did not exceed the GES threshold in the study area, while Cd in sediment samples exceeded the threshold at two stations. The content of Cu in the bottom sediments may show a warning sign as its concentrations exceed (in the Neva Estuary) and approach the suggested provisional threshold levels in multiple locations across the study area. Overall, the high ecological risk of sediment contamination by heavy metals for the offshore eastern Gulf of Finland area and the outer Neva Estuary was identified from the calculated potential ecological risk index. The heavy metals had a specific pattern of distribution in the study area as atmospheric deposition and waterborne inputs in different proportions are the main sources of these elements to the aquatic environment of the eastern Gulf of Finland.

The simulated accumulation pattern is indicating the most probable zones of riverine origin HS accumulation. Depending on the settling velocity, HS might disperse along the shoreline in the eastern Gulf of Finland much further from the initial release locations within the river estuary systems. Sedimentation of the medium size particles shows amounts growing from the outer part of the Neva Estuary to the east, highlighting the threat of increasing amounts of contaminants that are also visible from the available observations on heavy metals. This modelled gradient should be considered when planning monitoring activities in the eastern Gulf of Finland.

The evidenced occurrence of periodically oxygen-depleted sediments can partly explain the presence and preservation of studied organic pollutants at the observed levels in the region. The increase of deoxygenated zones over time becomes the factor contributing to the sustainment of long-standing environmental issues regarding HS in the future as well.

CRediT authorship contribution statement

Ivan Kuprijanov: Project administration, Supervision, Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Germo Väli:** Conceptualization, Software, Visualization, Writing – review & editing. **Andrey Sharov:** Investigation, Writing – review & editing. **Nadezhda Berezina:** Investigation, Writing – review & editing. **Taavi Liblik:** Writing – review & editing. **Urmas Lips:** Writing – review & editing, Funding acquisition. **Natalja Kolesova:** Writing – review & editing. **Jaakko Maanio:** Investigation. **Ville Junttila:** Investigation. **Inga Lips:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was conducted in the frames of the projects ER90 HAZLESS of the “Estonia-Russia Cross-Border Cooperation Program 2014 –2020” (https://hazless.msi.ttu.ee) and “Actions to evaluate and identify effective measures to reach GES in the Baltic Sea marine region (HELCOM ACTION)”. This study was also supported by the Estonian Research Council grant “The role of sub-mesoscale processes in structuring and large-scale dynamics of oceanographic fields” (grant number PRG602, 2020). This publication has been produced with the financial assistance of the Estonia – Russia Cross Border Cooperation Programme 2014-2020. The content of this publication is the sole responsibility of authors and can under no circumstances be regarded as reflecting the position of the Programme participating countries alongside with the European Union. The allocation of computing time on the HPC clusters by the TalTech and Tartu University is gratefully acknowledged. We thank two anonymous reviewers for their helpful and constructive

comments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112642.

References

Abraham, M., Westphal, L., Hand, I., Lerz, A., Jeschek, J., Bunke, D., Leipe, T., Schulz-Bull, D., 2017. TBT and its metabolites in sediments: survey at a German coastal site and the central Baltic Sea. Mar. Pollut. Bull. 121 (1–2), 404–410.

Adamo, P., Arienzo, M., Imperato, M., Naimo, D., Nardi, G., Stanzione, D., 2005. Distribution and partition of heavy metals in surface and sub-surface sediments of Naples city port. Chemosphere 61, 800–809.

Ærtebjerg, G., Andersen, J.H., Hansen, O.S., 2003. Nutrients and Eutrophication in Danish Marine Waters. A Challenge for Science and Management. National Environmental Research Institute, p. 126.

Alenius, P., Myrberg, K., Nekrasov, A., 1998. The physical oceanography of the Gulf of Finland: a review. Boreal Environ. Res. 3 (2), 97–125.

AMAP, 2017. AMAP Assessment 2016: Chemicals of Emerging Arctic Concern. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway xvi+353pp.

Antizar-Ladislao, B., 2008. Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment. A review. Environ. Int. 34, 292–308.

Arinushkina, E.V., 1970. Rukovodstvo po khimicheskomu analizu pochv (Manual on Soil Chemical Analysis). Mosk. Gos. Univ., Moscow (In Russian).

Aslam, S.N., Venzi, M.S., Venkatraman, V., Mikkelsen, Ø., 2020. Chemical assessment of marine sediments in vicinity of Norwegian fish farms – a pilot study. Sci. Total Environ. 732, 139130.

Barrick, R.C., Prahl, F.G., 1987. Hydrocarbon geochemistry of the Puget Sound region—III. Polycyclic aromatic hydrocarbons in sediments. Estuar. Coast. Shelf Sci. 25 (2), 175–191.

Bartnicki, J., Gusev, A., Aas, W., Gauss, M., Jonson, J.E., 2017. Atmospheric supply of nitrogen, cadmium, mercury, lead, and PCDD/Fs to the Baltic Sea in 2015. In: EMEP Report to HELCOM. https://www.emep.int/publ/helcom/2017/index.html.

Bauer, J.E., Capone, D.G., 1985. Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. Appl. Environ. Microbiol. 50 (1), 81–90.

Baumard, P., Budzinski, H., Michon, Q., Garrigues, P., Burgeot, T., Bellocq, J., 1998. Origin and bioavailability of PAHs in the Mediterranean Sea from mussel and sediment records. Estuar. Coast. Shelf Sci. 47 (1), 77–90.

Baumard, P., Budzinski, H., Garrigues, P., Narbonne, J.F., Burgeot, T., Michel, X., Bellocq, J., 1999. Polycyclic aromatic hydrocarbon (PAH) burden of mussels (Mytilus sp.) in different marine environments in relation with sediment PAH contamination, and bioavailability. Mar. Environ. Res. 47, 415–439.

Berezina, N.A., Gubelit, Y.I., Polyak, Y.M., Sharov, A.N., Kudryavtseva, V.A., Lubimtsev, V.A., Petukhov, V.A., Shigaeva, T.D., 2017. An integrated approach to the assessment of the eastern Gulf of Finland health: a case study of coastal habitats. J. Mar. Syst. 171, 159–171.

Berezina, N.A., Lehtonen, K.K., Ahvo, A., 2019. Coupled application of antioxidant defense response and embryo development in amphipod crustaceans in the assessment of sediment toxicity. Environ. Toxicol. Chem. 38 (9), 2020–2031.

Biselli, S., Bester, K., Hühnerfuss, H., Fent, K., 2000. Concentrations of the antifouling compound Irgarol 1051 and of organotins in water and sediments of German North and Baltic Sea marinas. Mar. Pollut. Bull. 40, 233–243.

Blankholm, H.P., Lidén, K., Kovačević, N., Angerbjörn, K., 2020. Dangerous food. Climate change induced elevated heavy metal levels in Younger Stone Age seafood in northern Norway. Quat. Int. 549, 74–83.

de Boer, J., van der Zande, T.E., Pieters, H., Ariese, F., Schipper, C.A., van Brummelen, T., Vethaak, A.D., 2001. Organic contaminants and trace metals in flounder liver and sediment from the Amsterdam and Rotterdam harbours and off the Dutch coast. J. Environ. Monit. 3 (4), 386–393.

Borg, H., Jonsson, P., 1996. Large-scale metal distribution in Baltic Sea sediments. Mar. Pollut. Bull. 32 (1), 8–21.

Brüggeman, J., Bolding, K., 2014. A general framework for aquatic biogeochemical models. Environ. Model. Softw. 61, 249–265.

Budzinski, H., Jones, I., Bellocq, J., Pierard, C., Garrigues, P., 1997. Evaluation of sediment contamination by polycyclic aromatic hydrocarbons in the Gironde estuary. Mar. Chem. 58, 85–97.

Burchard, H., Bolding, K., 2002. GETM: A General Estuarine Transport Model; Scientific Documentation. Technical Report EUR 20253. EN, European Commission, Ispra, Italy. https://op.europa.eu/en/publication-detail/-/publication/5506bf19-e076-4d4b-8648-dedd06efbb38.

Canadian Council of Ministers of the Environment, 2002. Canadian sediment quality guidelines for the protection of aquatic life: summary tables. Updated. In: Canadian Environmental Quality Guidelines. Canadian Council of Ministers of the Environment, Winnipeg, p. 1999.

Carolin, C.F., Kumar, P.S., Saravanan, A., Joshiba, G.J., Naushad, M., 2017. Efficient techniques for the removal of toxic heavy metals from aquatic environment: a review. J. Environ. Chem. Eng. 5, 2782–2799.

Chakraborty, P., Chakraborty, S., Jayachandran, S., Madan, R., Sarkar, A., Linsy, P., Nath, B.N., 2016. Effects of bottom water dissolved oxygen variability on copper and lead fractionation in the sediments across the oxygen minimum zone, western continental margin of India. Sci. Total Environ. 566, 1052–1061.

Conley, D.J., Carstensen, J., Aigars, J., Axe, P., Bonsdorff, E., Eremina, T., Haahti, B.M., Humborg, C., Jonsson, P., Kotta, J., Lannegren, C., 2011. Hypoxia is increasing in the coastal zone of the Baltic Sea. Environ. Sci. Technol. 45, 6777–6783.

Cornelissen, G., Pettersen, A., Nesse, E., Eek, E., Helland, A., Breedveld, G.D., 2008. The contribution of urban runoff to organic contaminant levels in harbour sediments near two Norwegian cities. Mar. Pollut. Bull. 56, 565–573.

Dafforn, K.A., Lewis, J.A., Johnston, E.L., 2011. Antifouling strategies: history and regulation, ecological impacts and mitigation. Mar. Pollut. Bull. 62 (3), 453–465.

De Luca, G., Furesi, A., Micera, G., Panzanelli, A., Piu, P.C., Pilo, M.I., Spano, N., Sanna, G., 2005. Nature, distribution and origin of polycyclic aromatic hydrocarbons (PAHs) in the sediments of Olbia harbor (Northern Sardinia, Italy). Mar. Pollut. Bull. 50, 1223–1232.

Díez, S., Ábalos, M., Bayona, J.M., 2002. Organotin contamination in sediments from the Western Mediterranean enclosures following 10 years of TBT regulation. Water Res. 36, 905–918.

Eklund, B., 2017. Review of the use of Ceramium tenuicorne growth inhibition test for testing toxicity of substances, effluents, products sediment and soil. Estuar. Coast. Shelf Sci. 195, 88–97.

El Nemr, A., El-Sadaawy, M.M., Khaled, A., Draz, S.O., 2013. Aliphatic and polycyclic aromatic hydrocarbons in the surface sediments of the Mediterranean: assessment and source recognition of petroleum hydrocarbons. Environ. Monit. Assess. 185, 4571–4589.

Falandysz, J., Albanis, T., Bachmann, J., Bettinett, I.R., Bochentin, I., Boti, V., Bristeau, S., Daehne, B., Dagnac, T., Galassi, S., 2006. Some chemical contaminant of surface sediments at the Baltic Sea coastal region with special emphasis on androgenic and anti-androgenic compounds. J. Environ. Sci. Health A 41, 2127–2162.

Fent, K., 2006. Worldwide occurrence of organotins from antifouling paints and effects in the aquatic environment. Handb. Environ. Chem 5, 71–100.

Fernex, F.E., Migon, C., Chisholm, J.R.M., 2001. Entrapment of pollutants in Mediterranean sediments and biogeochemical indicators of their impact. Hydrobiologia 450, 31–46.

Filipkowska, A., Kowalewska, G., Pavoni, B., 2014. Organotin compounds in surface sediments of the Southern Baltic coastal zone: a study on the main factors for their accumulation and degradation. Environ. Sci. Pollut. Res. 21 (3), 2077–2087.

Filipkowska, A.A., Kowalewska, G.G., Pavoni, B.B., Łęczyński, L.L., 2011. Organotin compounds in surface sediments from seaports on the Gulf of Gdańsk (southern Baltic coast). Environ. Monit. Assess. 182 (1–4), 455–466.

Galkus, A., Joksas, K., Stakeniene, R., Lagunaviciene, L., 2012. Heavy metal contamination of harbor bottom sediments. Pol. J. Environ. Stud. 21, 1583–1594.

Gilek, M., Björk, M., Broman, D., Kautsky, N., Kautsky, U., Näf, C., 1997. The role of the blue mussel, Mytilus edulis, in the cycling of hydrophobic organic contaminants in the Baltic proper. Ambio 202–209.

Gräwe, U., Wolff, J.O., 2010. Suspended particulate matter dynamics in a particle framework. Environ. Fluid Mech. 10, 21–39.

Gräwe, U., Holtermann, P., Klingbeil, K., Burchard, H., 2015. Advantages of vertically adaptive coordinates in numerical models of stratified shelf seas. Ocean Model 92, 56–68.

Hakanson, L., 1980. An ecological risk index for aquatic pollution control. A sedimentological approach. Water Res. 14, 975–1001.

Hallikainen, A., Airaksinen, R., Rantakokko, P., Vuorinen, P., Mannio, J., Lappalainen, A., Vihervuori, A., Vartiainen, T., 2008. Levels of organic tin compounds in Baltic Sea and Finnish fresh water fish. In: Evira Research Reports 6/2008. Finnish Food Safety Authority Evira, Helsinki, Finland, 69 p (in Finnish, Swedish and English abstract).

Han, T., Kang, S.-H., Park, J.-S., Lee, H.-K., Brown, M.T., 2008. Physiological responses of Ulva pertusa and U. armoricana to copper exposure. Aquat. Toxicol. 86, 176–184.

Heiri, O., Lotter, A.F., Lemcke, G., 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. J. Paleolimnol. 25, 101–110.

HELCOM, 2010. Maritime activities in the Baltic Sea – an integrated thematic assessment on maritime activities and response to pollution at sea in the Baltic Sea region. In: Baltic Sea Environment Proceedings, vol. 123. Helsinki Commission, Helsinki, pp. 1–64.

HELCOM, 2011. Fifth Baltic Sea Pollution Load Compilation (PLC-5). In: Baltic Sea Environment Proceedings, p. 128 (Helsinki Commission, Helsinki).

HELCOM, 2012. Development of a Set of Core Indicators: Interim Report of the HELCOM CORESET Project. PART B: Descriptions of the Indicators Baltic Sea Environment Proceedings 129B. http://www.helcom.fi/Lists/Publications/BSEP129B.pdf.

HELCOM, 2013. HELCOM Core Indicators: Final Report of the HELCOM CORESET Project. Baltic Sea Environment Proceedings. Helsinki Commission, Helsinki, p. 136.

HELCOM, 2018a. HELCOM Thematic Assessment of Hazardous Substances 2011–2016. Baltic Sea Environment Proceedings. Helsinki Commission, Helsinki, p. 157.

HELCOM, 2018b. HELCOM assessment on maritime activities in the Baltic Sea 2018. In: Baltic Sea Environment Proceedings. Helsinki Commission, Helsinki, p. 152.

HELCOM, 2018c. Metals (lead, cadmium and mercury). HELCOM core indicator report. Online. [08.09.2020]. https://helcom.fi/wp-content/uploads/2019/08/Metals-HELCOM-core-indicator-2018.pdf.

HELCOM, 2018d. Tributyltin TBT and imposex. HELCOM core indicator report. Online. [08.09.2020]. https://helcom.fi/wp-content/uploads/2019/08/Tributyltin-TBT-and-imposex-HELCOM-core-indicator-2018.pdf.

HELCOM, 2018e. PAH and metabolites. HELCOM core indicator report. Online. [08.09.2020]. https://helcom.fi/wp-content/uploads/2019/08/Polyaromatic-hydrocarbons-PAHs-and-their-metabolites-HELCOM-core-indicator-2018.pdf.

HELCOM, 2018f. Inputs of hazardous substances to the Baltic Sea. In: Baltic Sea Environment Proceedings 161. Online [14.01.2021]. https://helcom.fi/media/publications/BSEP162.pdf.
Hoch, M., 2001. Organotin compounds in the environment — an overview. Appl. Geochem. 16 (7–8), 719–743.
Hofmeister, R., Burchard, H., Beckers, J.M., 2010. Non-uniform adaptive vertical grids for 3D numerical ocean models. Ocean Model 33 (1–2), 70–86.
ICES DOME dataset, 2017/2019. ICES, Copenhagen.
Johansson, L., Ytreberg, E., Jalkanen, J.P., Fridell, E., Eriksson, K.M., Lagerström, M., Maljutenko, I., Raudsepp, U., Fischer, V., Roth, E., 2020. Model for leisure boat activities and emissions–implementation for the Baltic Sea. Ocean Sci. 16 (5), 1143–1163.
Jokšas, K., Stakėnienė, R., Raudonytė-Svirbutavičienė, E., 2019. On the effectiveness of tributyltin ban: distribution and changes in butyltin concentrations over a 9-year period in Klaipėda Port, Lithuania. Ecotoxicol. Environ. Saf. 183, 109515.
Kalnejais, L.H., Martin, W.R., Signell, R.P., Bothner, M.H., 2007. Role of sediment resuspension in the remobilization of particulate-phase metals from coastal sediments. Environ. Sci. Technol. 41 (7), 2282–2288.
Kalnejais, L.H., Martin, W.R., Bothner, M.H., 2010. The release of dissolved nutrients and metals from coastal sediments due to resuspension. Mar. Chem. 121 (1–4), 224–235.
Keith, L.H., 2015. The source of US EPA's sixteen PAH priority pollutants. Polycycl. Aromat. Compd. 35 (2–4), 147–160.
KEMI, 2006. KEMl Report 2/06: Chemical Substances in Antifouling Paints. Swedish Chemicals Agency, Sundbyberg, Sweden.
Kennish, M.J., 1997. Practical Handbook of Estuarine and Marine Pollution. CRC Press marine science series, United States of America, 524 p.
Khairy, M.A., Kolb, M., Mostafa, A.R., Anwar, E.F., Bahadir, M., 2009. Risk assessment of polycyclic aromatic hydrocarbons in a Mediterranean semi-enclosed basin affected by human activities (Abu Qir Bay, Egypt). J. Hazard. Mater. 170 (1), 389–397.
Knutzen, J., 1995. Effects on marine organisms from polycyclic aromatic hydrocarbons (PAH) and other constituents of waste water from aluminium smelters with examples from Norway. Sci. Total Environ. 163, 107–122.
Komárek, M., Ettler, V., Chrastný, V., Mihaljevič, M., 2008. Lead isotopes in environmental sciences: a review. Environ. Int. 34, 562–577.
Korpinen, S., Meski, L., Andersen, J.H., Laamanen, M., 2012. Human pressures and their potential impact on the Baltic Sea ecosystem. Ecol. Indic. 15 (1), 105–114.
Lagerström, M., Strand, J., Eklund, B., Ytreberg, E., 2017. Total tin and organotin speciation in historic layers of antifouling paint on leisure boat hulls. Environ. Pollut. 220, 1333–1341.
Latõšov, E., Volkova, A., Hlebnikov, A., Siirde, A., 2018. Technical improvement potential of large district heating network: application to the Case of Tallinn, Estonia. Energy Procedia 149, 337–344.
Lee, C.C., Hsieh, C.Y., Tien, C.J., 2006. Factors influencing organotin distribution in diferent marine environmental compartments, and their potential health risk. Chemosphere 65, 547–559.
Legorburu, I., Rodríguez, J.G., Valencia, V., Solaun, O., Borja, Á., Millán, E., Galparsoro, I., Larreta, J., 2014. Sources and spatial distribution of polycyclic aromatic hydrocarbons in coastal sediments of the Basque Country (Bay of Biscay). Chem. Ecol. 30, 701–718.
Lehtonen, K.K., Bignert, A., Bradshaw, C., Broeg, K., Schiedek, D., 2017. Chemical pollution and ecotoxicology. In: Biological Oceanography of the Baltic Sea. Springer, Dordrecht, pp. 547–587.
Liblik, T., Lips, U., 2017. Variability of pycnoclines in a three-layer, large estuary: the Gulf of Finland. Boreal Environ. Res. 22, 27–47.
Liblik, T., Väli, G., Lips, I., Lilover, M.-J., Kikas, V., Laanemets, J., 2020. The winter stratification phenomenon and its consequences in the Gulf of Finland, Baltic Sea. Ocean Sci. 16, 1475–1490.
Liiv, S., Kaasik, M., 2004. Trace metals in mosses in the Estonian oil shale processing region. J. Atmos. Chem. 49 (1), 563–578.
Lindström, G., Pers, C.P., Rosberg, R., Strömqvist, J., Arheimer, B., 2010. Development and test of the HYPE (Hydrological Predictions for the Environment) model – a water quality model for different spatial scales. Hydrol. Res. 41 (3–4), 295–319.
Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manag. 19, 81–97.
Macdonald, D.D., Carr, R.S., Calder, F.D., Long, E.R., Ingersoll, C.G., 1996. Development and evaluation of sediment quality guidelines for Florida coastal waters. Ecotoxicology 5, 253–278.
Männik, A., Merilain, M., 2007. Verification of different precipitation forecasts during extended winter-season in Estonia. In: HIRLAM Newsl. 65−70. Swedish Meteorol. Hydrol. Institute.
Mannio, J., Lehtonen, K., Jørgensen, K., Kankaanpää, H., Korneev, O., Mehtonen, J., Roots, O., Vallius, H., Vuorinen, P., Äystö, L., Fedorova, N., Keinänen, M., Lukki, T., Lyachenko, O., Rybalko, A., Salo, S., Söderström, S., Turja, R., Zhakovskaja, Z., 2016. Hazardous substances. In: Raateoja, Mika, Setälä, Outi (Eds.), The Gulf of Finland Assessment, Reports of the Finnish Environment Institute, 27, pp. 154–189.
Marston, C.P., Pereira, C., Ferguson, J., Fischer, K., Hedstrom, O., Dashwood, W.M., Baird, W., 2001. Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumor initiation, PAH_DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. Carcinogenesis 22, 1077–1086.
Martınez, C.E., Motto, H.L., 2000. Solubility of lead, zinc and copper added to mineral soils. Environ. Pollut. 107 (1), 153–158.
McGrath, J.A., Joshua, N., Bess, A.S., Parkerton, T.F., 2019. Review of polycyclic aromatic hydrocarbons (PAHs) sediment quality guidelines for the protection of benthic life. Integr. Environ. Assess. Manag. 15 (4), 505–518.
Means, J.C., 1998. Compound-specific gas chromatographic/mass spectrometric analysis of alkylated and parent poly cyclic aromatic hydrocarbons in waters, sediments, and aquatic organisms. J. AOAC Int. 81 (3), 657–672.
Menzie, C.A., Potocki, B.B., Santodonato, J., 1992. Exposure to carcinogenic PAHs in the environment. Environ. Sci. Technol. 26 (7), 1278–1284.
Michel, P., Averty, B., Andral, B., Chiffoleau, J.F., Galgani, F., 2001. Tributyltin along the coasts of Corsica (Western Mediterranean): a persistent problem. Mar. Pollut. Bull. 42 (11), 1128–1132.
de Mora, S.J., Pelletier, E., 1997. Environmental tributyltin research: past, present, future. Environ. Sci. Technol. 18, 1169–1177.
Morillo, E., Romero, A.S., Maqueda, C., Madrid, L., Ajmone-Marsan, F., Grcman, H., Davidson, C.M., Hursthouse, A.S., Villaverde, J., 2008. Soil pollution by PAHs in urban soils: a comparison of three European cities. J. Environ. Monit. 9, 1001–1008.
Neff, J.M., 2004. Bioaccumulation in Marine Organisms. Effect of Contaminants From Oil Well Produced Water. Elsevier, Amsterdam, London, Tokio, 241–318 pp.
Nikinmaa, M., 2013. Climate change and ocean acidification—interactions with aquatic toxicology. Aquat. Toxicol. 126, 365–372.
Nikulina, A., Polovodova, I., Schönfeld, J., 2008. Foraminiferal response to environmental changes in Kiel Fjord, SW Baltic Sea. eEarth 3, 37–49.
Nor, Y.M., 1987. Ecotoxicity of copper to aquatic biota: a review. Environ. Res. 43 (1), 274–282.
Norkko, A., Bonsdorff, E., 1996. Rapid zoobenthic community responses to accumulations of drifting algae. Mar. Ecol. Prog. Ser. 131, 143–157.
Nour, H.E.S., 2019. Assessment of heavy metals contamination in surface sediments of Sabratha, Northwest Libya. Arab. J. Geosci. 12.
Nour, H.E.S., 2020. Distribution and accumulation ability of heavy metals in bivalve shells and associated sediment from Red Sea coast, Egypt. Environ. Monit. Assess. 192.
Nour, H.E.S., Nouh, E.S., 2020. Comprehensive pollution monitoring of the Egyptian Red Sea coast by using the environmental indicators. Environ. Sci. Pollut. Res. 27, 28813–28828.
Okoro, H.K., Fatoki, O.S., Adekola, F.A., Ximba, B.J., Snyman, R.G., 2011. Sources environmental levels and toxicity of organotin in marine environment: a review. Asian J. Chem. 23 (2), 473–482.
Omae, I., 2003. Organotin antifouling paints and their alternatives. Appl. Organomet. Chem. 17, 81–105.
Osinski, R.D., Radtke, H., 2020. Ensemble hindcasting of wind and wave conditions with WRF and WAVEWATCH III® driven by ERA5. Ocean Sci. 16, 355–371.
OSPAR, 1998. Report of the Third OSPAR Workshop on Ecotoxicological Assessment Criteria (EAC).The Hague, 25-29 November 1996. Oslo and Paris Commissions.
Page, D.S., Boehm, P.D., Douglas, G.S., Bence, A.E., Burns, W.A., Mankiewicz, P.J., 1999. Pyrogenic polycyclic aromatic hydrocarbons in sediments record past human activity: a case study in Prince William Sound, Alaska. Mar. Pollut. Bull. 38, 247–260.
Panov, V.E., Alimov, A.F., Golubkov, S.M., Orlova, M.I., Telesh, I.V., 2002. Environmental problems and challenges for coastal zone management in the Neva Estuary (Eastern Gulf of Finland). In: Baltic Coastal Ecosystems. Springer, Berlin, Heidelberg, pp. 171–184.
Peng, S.H., Wang, W.X., Li, X., Yen, Y.F., 2004. Metal partitioning in river sediments measured by sequential extraction and biomimetic approaches. Chemosphere 57 (8), 839–851.
Pikkarainen, A.L., 2004. Polycyclic aromatic hydrocarbons in Baltic sea sediments. Polycycl. Aromat. Compd. 24, 667–679.
Pitkänen, H., Lehtoranta, J., Räike, A., 2001. Internal nutrient fluxes counteract decreases in external load: the case of the estuarial eastern Gulf of Finland, Baltic Sea. AMBIO J. Hum. Environ. 30, 195–201.
Pohl, C., Löffler, A., Schmidt, M., Seifert, T., 2006. A trace metal (Pb, Cd, Zn, Cu) balance for surface waters in the eastern Gotland Basin, Baltic Sea. J. Mar. Syst. 60, 381–395.
Qing, X., Yutong, Z., Shenggao, L., 2015. Assessment of heavy metal pollution and human health risk in urban soils of steel industrial city (Anshan), Liaoning, Northeast China. Ecotoxicol. Environ. Saf. 120, 377–385.
R Core Team, 2020. R: A Language and Environment for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Radke, B., Łęczyński, L., Wasik, A., Namieśnik, J., Bolalek, J., 2008. The content of butyl- and phenyltin derivatives in the sediment from the Port of Gdansk. Chemosphere 73, 407–414.
Raukas, A., 2010. Sustainable development and environmental risks in Estonia. Agron. Res. 8, 351–356.
Remeikaitė-Nikienė, N., Garnaga-Budrė, G., Lujanienė, G., Jokšas, K., Stankevičius, A., Malejevas, V., Barisevičiūtė, R., 2018. Distribution of metals and extent of contamination in sediments from the south-eastern Baltic Sea (Lithuanian zone). Oceanologia 60 (2), 193–206.
Rieuwerts, J., Farago, M., Cikrt, M., Bencko, V., 1999. Heavy metal concentrations in and around households near a secondary lead smelter. Environ. Monit. Assess. 58, 317–335.
Rigaud, S., Radakovitch, O., Couture, R.M., Deflandre, B., Cossa, D., Garnier, C., Garnier, J.M., 2013. Mobility and fluxes of trace elements and nutrients at the sediment–water interface of a lagoon under contrasting water column oxygenation conditions. Appl. Geochem. 31, 35–51.
Rodriguez-Cea, A., Rodriguez-Gonzalez, P., Garcia Alonso, J.I., 2016. Study of the degradation of butyltin compounds in surface water samples under different storage conditions using multiple isotope tracers and GC-MS/MS. Environ. Sci. Pollut. Res. Int. 23 (5), 4876–4885.
Rodriguez-Gonzalez, P., Bouchet, S., Monperrus, M., Tessier, E., Amouroux, D., 2013. In situ experiments for element species-specific environmental reactivity of tin and

mercury compounds using isotopic tracers and multiple linear regression. Environ. Sci. Pollut. Res. 20, 1269–1280.
Roots, O., Roose, A., 2013. Hazardous substances in the aquatic environment of Estonia. Chemosphere 93 (1), 196–200.
Ryabchuk, D., Vallius, H., Zhamoida, V., Kotilainen, A.T., Rybalko, A., Malysheva, N., Deryugina, N., Sukhacheva, L., 2017. Pollution history of Neva Bay bottom sediments (eastern Gulf of Finland, Baltic Sea). Baltica 30 (1).
Rybalko, A.E., Fedorova, N.K., 2008. Bottom sediments of the Neva estuary and its contamination under influence of anthropogenic processes. In: Alimov, A.F., Golubkov, S.M. (Eds.), Ecosystem of the Neva Estuary: Biological Diversity and Ecological Problems. KMK, St. Petersburg—Moscow, 39–58 pp. (in Russian).
Sahlin, S., Ågerstrand, M., 2018. Copper in Sediment EQS Data Overview. ACES Report Number 28. Department of Environmental Science and Analytical Chemistry (ACES) Stockholm University.
SEDGOF, 2016. "Hinnangu andmine merekeskkonna ökosüsteemipõhiseks korraldamiseks Soome lahe merepõhja ja setete näitel" (SEDGOF). Aruanne, Tallinn. https://www.kik.ee/sites/default/files/uuringud/aruanne_sedgof_30.06.2016.pdf.
Senze, M., Kowalska-Góralska, M., Pokorny, P., Dobicki, W., Polechoński, R., 2015. Accumulation of heavy metals in bottom sediments of Baltic Sea catchment rivers affected by operations of petroleum and natural gas mines in Western Pomerania, Poland. Pol. J. Environ. Stud. 24 (5), 2167–2175.
Short, J.W., Irvine, G.V., Mann, D.H., Maselko, J.M., Pella, J.J., Lindeberg, M.R., Payne, J.R., Driskell, W.B., Rice, S.D., 2007. Slightly weathered Exxon Valdez oil persists in gulf of Alaska beach sediments after 16 years. Environ. Sci. Technol. 41, 1245–1250.
Simpson, S.L., Batley, G.B., Chariton, A.A., 2013. Revision of the ANZECC/ARMCANZ Sediment Quality Guidelines. CSIRO Land and Water Science Report 08/07. CSIRO Land and Water.
Srivastava, P., Sreekrishnan, T.R., Nema, A.K., 2017. Degradation of low-molecular-weight PAHs: naphthalene, acenaphthylene, phenanthrene, and fluorene. J. Hazard. Toxic. Radioact. Waste 21 (4), 04017008.
Stakėnienė, R., Jokšas, K., Galkus, A., Raudonytė-Svirbutavičienė, E., 2016. Aliphatic and polycyclic aromatic hydrocarbons in the bottom sediments from Klaipėda Harbour, Lithuania (Baltic Sea). Chem. Ecol. 32, 357–377.
Stark, J.S., Riddle, M.J., Snape, I., Scouller, R.C., 2003. Human impacts in Antartic marine soft-sediment assemblages: correlations between multivariate biological patterns and environmental variables at Casey Station. Estuar. Coast. Shelf Sci. 56, 717–734.
Stemmler, I., Lammel, G., 2009. Cycling of DDT in the global environment 1950–2002: World ocean returns the pollutant. Geophys. Res. Lett. 36 (24).
Stogiannidis, E., Laane, R., 2015. Source characterization of polycyclic aromatic hydrocarbons by using their molecular indices: an overview of possibilities. In: Reviews of Environmental Contamination and Toxicology. Springer, Cham, 49-133 pp.
Stoicescu, S.T., Lips, U., Liblik, T., 2019. Assessment of eutrophication status based on sub-surface oxygen conditions in the Gulf of Finland (Baltic Sea). Front. Mar. Sci. 6, 54.
Sunday, A.O., Alafara, B.A., Oladele, O.G., 2012. Toxicity and speciation analysis of organotin compounds. Chem. Speciat. Bioavailab. 24 (4), 216–226.
Suzdalev, S., Gulbinskas, S., 2014. Total petroleum hydrocarbons in surface sediments of the Lithuanian coastal area of the Baltic Sea. Baltica 27.
Suzdalev, S.S., Gulbinskas, S.S., Blažauskas, N.N., 2015. Distribution of tributyltin in surface sediments from transitional marine-lagoon system of the south-eastern Baltic Sea, Lithuania. Environ. Sci. Pollut. Res. 22 (4), 2634–2642.
Swedish Chemicals Agency, 1993. Antifouling products: pleasure boats, commercial vessels, nets, fish cages and other underwater equipment. In: Report No.: 2/93.
Takeuchi, I., Takahashi, S., Tanabe, S., Miyazaki, N., 2004. Butyltin concentrations along the Japanese coast from 1997 to 1999 monitored by Caprella spp.(Crustacea: Amphipoda). Mar. Environ. Res. 57 (5), 397–414.
Tista, M., Gager, M., Haider, S., Klösch, N., Thielen, P., 2014. European Union Emission Inventory Report 1990–2012 Under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). European Environment Agency.
Umlauf, L., Burchard, H., 2005. Second-order turbulence closure models for geophysical boundary layers. A review of recent work. Cont. Shelf Res. 25 (7–8), 795–827.
Üveges, M., Rodríguez-González, P., Alonso, J.I.G., Sanz-Medel, A., Fodor, P., 2007. Isotope dilution analysis mass spectrometry for the routine measurement of butyltin compounds in marine environmental and biological samples. Microchem. J. 8 5 (1), 115–121.
Väli, G., Meier, M., Placke, M., Dieterich, C., 2019. River Runoff Forcing for Ocean Modeling Within the Baltic Sea Model Intercomparison Project, p. 113. https://doi.org/10.12754/msr-2019-0113.
Vallius, H., 2014. Heavy metal concentrations in sediment cores from the northern Baltic Sea: declines over the last two decades. Mar. Pollut. Bull. 79 (1–2), 359–364.
Viglino, L., Pelletier, É., St.-Louis, R., 2004. Highly persistent butyltins in northern marine sediments: a long-term threat for the Saguenay Fjord (Canada). Environ. Toxicol. Chem. 23 (11), 2673–2681.
Waite, M.E., Waldock, M.J., Thain, J.E., Smith, D.J., Milton, S.M., 1991. Reductions in TBT concentrations in UK estuaries following legislation in 1986 and 1987. Mar. Environ. Res. 32, 89–111.
Wang, Z., Fingas, M., Shu, Y.Y., Sigouin, L., Landriault, M., Lambert, P., Turpin, R., Campagna, P., Mullin, J., 1999. Quantitative characterization of PAHs in burn residue and soot samples and differentiation of pyrogenic PAHs from petrogenic PAHs– the 1994 mobile burn study. Environ. Sci. Technol. 33 (18), 3100–3109.
WHO (World Health Organization), 1989. IARC Monographs, vol. 46. International Agency for Research on Cancer, Lyon, France (41–155 pp).
Xie, Z.C., Wong, N.C., Qian, P.Y., Qiu, J.W., 2005. Responses of polychaete Hydroides elegans life stages to copper stress. Mar. Ecol. Prog. Ser. 285, 89–96.
Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchel, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. Org. Geochem. 33, 489–515.
Zhurbas, V., Väli, G., Golenko, M., Paka, V., 2018. Variability of bottom friction velocity along the inflow water pathway in the Baltic Sea. J. Mar. Syst. 184, 50–58.

Publication II

Kolesova, N., Sildever, S., Strode, E., Berezina, N., Sundelin, B., Lips, I., Kuprijanov, I., Buschmann, F., Gorokhova, E. (2024). **Linking contaminant exposure to embryo aberrations in sediment-dwelling amphipods: a multi-basin field study in the Baltic Sea.** Ecol. Indic. 160. https://doi.org/10.1016/j.ecolind.2024.111837

Ecological Indicators 160 (2024) 111837

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Linking contaminant exposure to embryo aberrations in sediment-dwelling amphipods: a multi-basin field study in the Baltic Sea

N. Kolesova[a,*], S. Sildever[a], E. Strode[b], N. Berezina[c], B. Sundelin[d], I. Lips[a,e], I. Kuprijanov[a], F. Buschmann[a], E. Gorokhova[d,*]

[a] Department of Marine Systems, Tallinn University of Technology, Estonia
[b] Latvian Institute of Aquatic Ecology, Latvia
[c] Zoological Institute, Russian Academy of Sciences, Russia
[d] Department of Environmental Science, Stockholm University, Sweden
[e] EuroGOOS AISBL, Belgium

A R T I C L E I N F O

Keywords:
Monoporeia affinis
Reproductive disorders
Embryo aberrations
Baltic Sea
Contaminants

A B S T R A C T

Embryo development of sediment-dwelling amphipod *Monoporeia affinis* is sensitive to contaminant exposure. Therefore, embryo aberrations in gravid females are used to detect the biological effects of contaminant exposure in the Baltic Sea benthic habitats. The indicator based on the aberration frequencies in wild populations (ReproIND) is currently used for environmental status assessment within the Marine Strategy Framework Directive, Descriptor 8.2. However, so far, it has mainly been applied in the Bothnian Sea (BoS) and the Western Gotland Basin (WGB), where it was found to respond to contaminant pressure and non-chemical environmental factors, such as temperature.

To expand the applicability of the indicator to other Baltic Sea basins, we used field data from the gulfs of Finland and Riga, BoS, and WGB to investigate the relationships between reproductive disorders and contaminants and environmental factors, thus evaluating the indicator suitability in these areas. Despite the natural variability of the environments and contaminant distribution across and within the basins, we found that high concentrations of contaminants, e.g. metals, PAHs, and PCBs, contribute significantly to the embryo aberrations in *M. affinis*. These findings support ReproIND applicability in the Baltic Sea and, perhaps, in other marine areas.

1. Introduction

The Baltic Sea is a semi-enclosed coastal sea with a limited water exchange, which makes it vulnerable to chemical pollution (Schneider et al., 2000, Jędruch et al., 2017, HELCOM, 2021). Despite massive efforts to reduce the input of hazardous substances, the contamination levels are still above acceptable levels (Löf et al., 2016a, HELCOM, 2018c, Kuprijanov et al., 2021). Intensive anthropogenic activities in the region are responsible for a broad spectrum of pollutants that reach the Baltic Sea, mainly through river runoff and atmospheric deposition (HELCOM, 2018a). Due to the slow water exchange and the long persistence of some contaminants, hazardous substances can be trapped in the sediments for long periods, leading to chronic exposure and adverse effects in biota (Strand & Asmund, 2003, Viglino et al., 2004, Cornelissen et al., 2008, Tansel et al., 2011). Effects caused by contaminants can be detected across different levels of biological organization, with measurable responses in animal biochemistry, physiology, reproduction, and behavior (Sundelin et al., 2000, Turja et al., 2014b, Kholodkevich et al., 2017, Pérez and Hoang, 2017, Podlesińska and Dąbrowska, 2019, Gorokhova et al., 2010, 2020, Berezina et al., 2022).

In the European Union, marine environment protection is coordinated by the Marine Strategy Framework Directive (MSFD), which aims to establish and maintain the good environmental status (GES) of marine ecosystems (European Commission, 2008). The Helsinki Commission (HELCOM) is responsible for MSFD implementation in the Baltic Sea region. It also serves as a platform for assessing and monitoring the status of the marine environment for establishing and maintaining GES. The evaluation of the marine environmental status involves eleven descriptors, including Descriptor 8 (D8), which focuses on the levels and impacts of the environmental pollution load (European Commission, 2008). The goal of the D8 assessment is to maintain contaminant

* Corresponding authors.
E-mail addresses: natalja.kolesova@taltech.ee (N. Kolesova), elena.gorokhova@aces.su.se (E. Gorokhova).

https://doi.org/10.1016/j.ecolind.2024.111837
Received 7 July 2023; Received in revised form 28 February 2024; Accepted 29 February 2024
Available online 13 March 2024

concentrations below levels that cause pollution effects. Biological effects caused by contaminants are assessed through Criteria D8C2, which considers population abundance and health, community composition, and habitat condition. Currently, the assessment of the Baltic Sea environmental status primarily relies on Criteria D8C1, which sets concentration thresholds for selected contaminants in water, sediment, and biota matrices, whereas only a few biological effect indicators are included in the assessments (HELCOM, 2010, 2018c, 2023).

The biological effect indicators allow quantifying the influence of contaminants on biota (Lam & Gray, 2003, Martín-Díaz et al., 2004). Therefore, establishing relationships between the pressures (i.e., concentrations of environmentally relevant contaminants and their mixtures) and effect indicators is a pre-requisite for the design, selection, and quality assessment under Criteria D8C2 and subsequent integration of the chemical and biological indicators for the overall D8 assessment (Queirós et al., 2016, Lyons et al., 2017). Moreover, the confounding factors need to be recognized and, if possible, accounted for in the indicator-based assessment to ensure that the regional threshold values and assessment criteria are ecologically meaningful (Gorokhova et al., 2010, Strode et al., 2023). Considering that environmental variables, e.g., the sediment grain size, temperature, oxygenation, and total organic carbon (TOC) content, are well-recognized drivers of the distribution and bioavailability of organic contaminants and metals in sediments (Tanner et al., 1993, Li et al., 2022), these factors should be evaluated for their potential contribution for the indicator variability in the target area.

Different species of amphipods are widely used in sediment bioassay tests (Podlesińska and Dąbrowska, 2019). The indicator ReproIND, *Reproductive disorders: malformed embryos of amphipods*, is one of the few biological effect indicators used for assessing the contaminant impacts *in situ* (HELCOM, 2023; HELCOM, 2018b). The indicator was developed for amphipod species *Monoporeia affinis* and *Pontoporeia femorata* and since 1994 used in the Swedish Marine Monitoring Programme (SNMMP) for the assessment of the contaminant effects in the Swedish coastal waters, the Bothnian Sea (BoS), the Quarken, the Northern Baltic Proper, and the Western Gotland Basin (WGB) (HELCOM, 2018b). ReproIND can be applied to other amphipod species that share similar reproduction biology, even if their habitat preferences differ (Sundelin et al., 2008b; HELCOM, 2018b).

The embryo aberration frequency is informative as it reflects the sensitivity of amphipod embryos to contaminant exposure during their development within the brood pouch, which lasts for weeks to months. Furthermore, it provides insights into female exposure over an extended period of up to two years, encompassing growth, maturation, and oogenesis (Löf et al., 2016a) . Several laboratory studies have reported the increased frequency of aberrant embryos in amphipods (Eriksson et al., 1996, Sundelin et al., 2008b, Berezina et al., 2019, 2022). However, demonstrating the linkage between pollution and biological effects indicators in the field is more challenging due to the simultaneous influence of several factors (McCarty & Mackay, 1993, Martín-Díaz et al., 2004). In the Baltic Sea, a higher frequency of aberrant embryos was found in contaminated than reference sites and decreased with a distance from the point sources (Sundelin & Eriksson, 1998, Reutgard et al., 2014). Also, in *M. affinis* population, the frequency of females with aberrant embryos showed a positive correlation with sediment concentrations of cadmium (Cd), polychlorinated biphenyls (PCBs), and chlorinated hydrocarbons (PAHs) (Löf et al., 2016a). However, the field evidence linking amphipod embryo aberrations to contaminants has been predominantly limited to regional studies, with a restricted range of environmental factors and contaminant distribution considered (Reutgard et al., 2014, Löf et al., 2016a, Berezina et al., 2017, Strode et al., 2017). To effectively apply ReproIND in the environmental assessment of different Baltic subbasins, obtaining a broader geographic coverage of embryo aberration occurrence across diverse environmental settings is crucial.

Here, we established relationships between embryo aberrations in *M. affinis* and hazardous substances across several subbasins in the Baltic Sea, where this species is abundant and used as a sentinel species to monitor the biological effects of contaminants (e.g., Löf et al., 2016a) and macrobenthic diversity (e.g., Raymond et al., 2021). Moreover, we also addressed the effects of the ecologically relevant confounding factors, i.e., bottom depth, temperature, oxygen, sediment grain size, and TOC, on the contaminants and the reproductive responses. Our findings validate the ReproIND indicator based on field data and expand its operationalization in the Baltic Sea, contributing to the comprehensive biological effect monitoring tools and supporting the Baltic Sea Action Plan (BSAP) (HELCOM, 2021).

2. Materials and methods

2.1. Sampling

Gravid *M. affinis* females were collected in four subbasins in the Baltic Sea (Fig. 1). Samples from GoF (14 sites) and GoR (3 sites) were a part of pilot projects between 2016 and 2021, whereas BoS (5 sites) and WGB (3 sites) data were collected within the Effect Screening Study (ESS; 2017–2018) coordinated by the Swedish Environmental Protection Agency. The sampling sites (25 in total, visited from December to March) represented both contaminated and relatively uncontaminated (i.e., without known point sources) sediments (Fig. 1).

Sediments and amphipods were collected with a bottom sled collecting the uppermost sediment layer (Blomqvist and Lundgren, 1996) in BoS and WGB, and van Veen sediment grab (sample area of 0.1 m^2) in GoF and GoR. The surface sediment layer (up to 5 cm) from the van Veen grab and mixed sediment from the sled samples were used for contaminant analysis and granulometry. After that, the sediment was sieved through 0.5–1 mm mesh size; gravid females were gently separated and kept alive in the seawater at the same temperature as the ambient environment ($< +4$ °C) until laboratory analyses. Salinity, temperature, and dissolved oxygen concentrations were measured from the near-bottom water layer (Table S1). For BoS and WGB sites, the temperatures (°C) within two weeks of the sampling occasion were extracted from the Swedish (Swedish National Oceanographic Data Centre at the Swedish Hydrological and Meteorological Institute, data available at: https://sharkweb.smhi.se).

2.2. Chemical analysis

Collected sediments were analyzed for heavy metals (As, Pb, Cd, Cr, Cu, Ni, Hg, Zn; mg kg^{-1}DW), polycyclic aromatic hydrocarbons (PAHs; ng g^{-1}DW), polychlorinated biphenyls (PCBs; ng g^{-1}DW), and butyltins (BTs; ng g^{-1}DW) (Table S2). In addition, the sediment grain size (%) and total organic carbon (TOC, %) were also analyzed (Table S1). Analysis of contaminants from sediment samples was outsourced and conducted in chemical laboratories in Estonia, Russia, Latvia, Germany, and Sweden (Table S3).

2.3. Embryo analysis

The embryo analysis was conducted using a stereomicroscope and following the methodology established by Sundelin et al. (2008b). The number of analyzed gravid females was recorded for each sampling site (Table S4). In each female, fecundity (eggs $female^{-1}$), embryo development stage (from 1 to 9; Stage) (Sundelin et al., 2008b), and embryo aberrations following the classification summarized by Löf et al. (2016a) were recorded. Different aberration types (Malf: malformed embryos, Membr: embryos with membrane damage, AD: embryos with arrested development, DE: dead eggs, and DB: dead brood) were used for calculating the ReproIND (HELCOM, 2018b). The indicator consists of two components calculated for each station: the proportion of aberrant embryos (%AbEmb; a sum of Malf, Membr, AD, and DE divided by the total number of the examined embryos) and the proportion of females

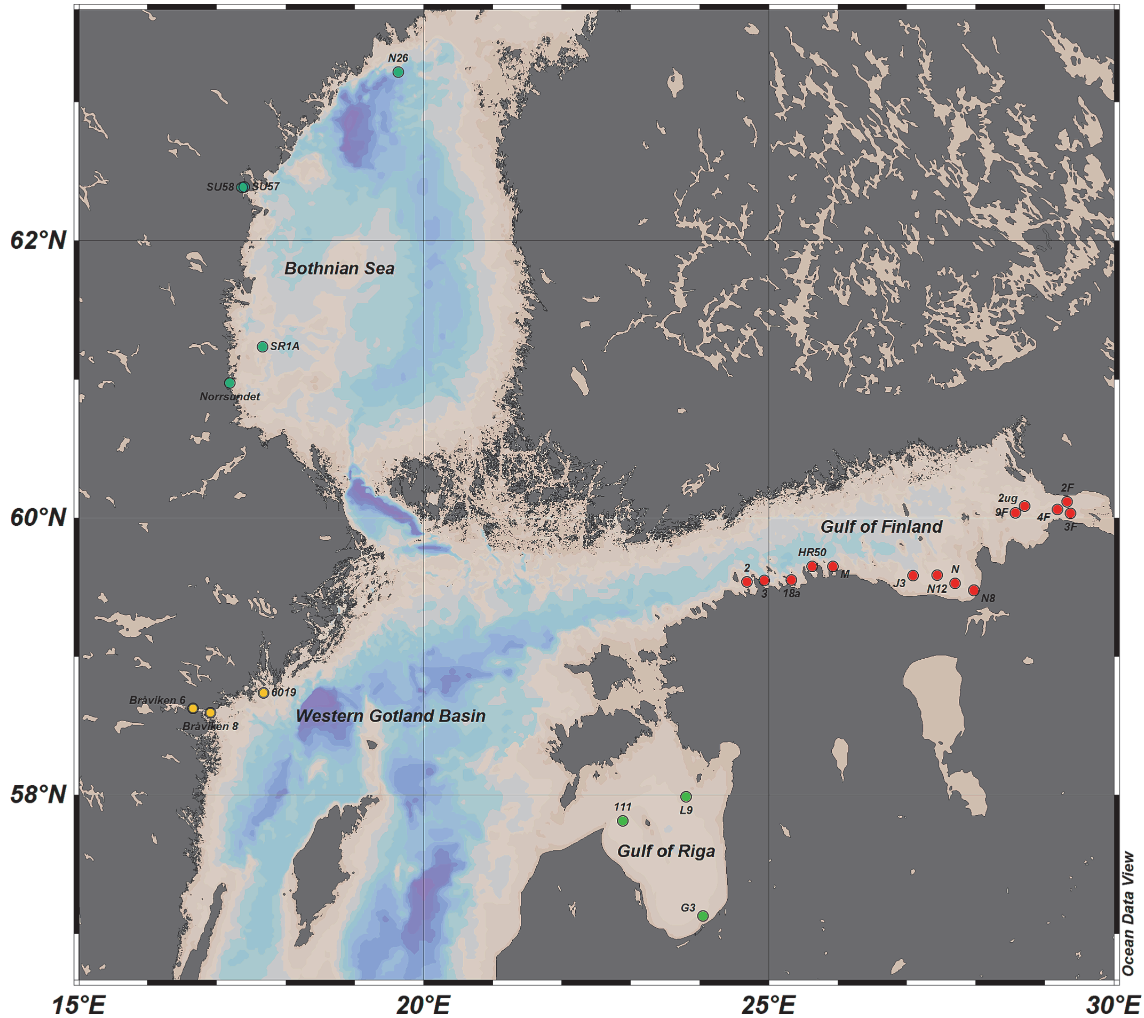

Fig. 1. Sampling locations for *M. affinis* and sediment samples in the Gulf of Finland (GoF): filled red circles, the Gulf of Riga (GoR): green, Bothnian Sea (BoS): blue, and the Western Gotland Basin (WGB): yellow.

with more than one aberrant embryo (%Fem > 1; the sum of gravid females carrying more than one aberrant embryo divided by the total number of the examined females).

2.4. Data analysis

2.4.1. Chemical data

For the total assessment of the metal contamination, we used the Tomlinson Pollution Load Index (PLI; Tomlinson et al., 1980) calculated as a geometric average of contamination factors for the nine metals (As, Cd, Co, Cr, Cu, Hg, Ni, Pb and Zn) measured in all data sets and used Swedish regional reference values for these metals in sediment (Havs- och vattenmyndigheten, 2018). The total concentrations of the 15 PAHs calculated as the sum of individual compounds (PAHs), the sum of four low-molecular-weight PAHs (LmPAHs: Phe, Ant, Flu, Pyr), and the sum of eight high-molecular-weight carcinogenic PAHs (HmPAHs: BaA, Chr, BbF, BkF, BaP, Inp, BgP, DBA) were used as descriptors of PAH contamination. For PCBs and BTs concentrations, the sum of congeners within each group was calculated. The concentrations below the limit of quantification (LOQ) were included in the univariate analysis as if they were true observations with a zero value. Due to the differences in LOQ among the analytical laboratories, we avoided replacing the non-detects with a fractional value of LOQ. The variables with > 60 % of the non-detects were omitted from the regression analysis.

2.4.2. Statistical evaluation

All *p* values presented are two-tailed, with $p < 0.05$ considered significant for all statistical tests. When relevant, the numerical variables were assessed for normality using the Shapiro-Wilk test. The numerical variables were presented as the mean ± standard deviation if the distribution was normal or as a median and interquartile range if the distribution was skewed. Reproductive aberrations were presented as frequencies (proportions), and the differences across the basins were evaluated using Fisher's exact test. Differences in sediment grain size (clay, sand, silt), TOC concentrations, and other environmental parameters (oxygen, salinity, and temperature) across the subbasins were evaluated using Kruskal-Wallis tests in R (R Core Team, 2024); here, we used non-parametric tests due to the lack of homogeneity in the variances and different number of sites across the subbasins.

2.4.3. Multivariate analyses

Multivariate ordination techniques were used for grouping the sampling sites according to their contaminant profiles and identification of the associated variables. The missing data for contaminants was replaced using EM (maximum expectation likelihood) algorithm, which assumes a multi-normal distribution model for the data (Dempster et al., 1977) with 1000 permutations as implemented in Primer 7 v.7.0.21 software (Anderson, 2017). The environmental and contaminant data were log-transformed and normalized to avoid misclassification due to the differences in data dimensionality (Anderson et al., 2008). As a pre-treatment transformation, the environmental and contaminant variables were log-transformed of log $(x + n)$, where n is a small number compared to the measured value. The transformed data were subjected to zero mean and unit variance normalization (z-scores), which were used in correlation and regression analyses and for the resemblance matrices in the multivariate analysis.

For visual data exploration, we used FreeViz, a free software including an optimization method that finds linear projection and associated scatterplots based on gradient descent modeling (Demšar et al., 2007). FreeViz separates instances of a different class evaluated through mean scores and graphical optimization for compaction and separation between instances of the same class. Also, a hierarchical cluster analysis (HCA) was performed on the contaminant data to identify groups of sites that had similar composition and concentrations of the contaminants. A classification scheme using the Euclidean distance for similarity measures between sites was performed. Ward's method was used for the establishment of the links between the sites to improve the distinctive power of the classification (Güler et al., 2002). Once the clusters were identified, we conducted a comparison of reproductive aberration frequencies across these clusters using an unpaired t-test following Box-Cox transformation of the frequency data to stabilize variances. This statistical analysis aimed to assess whether a general response to the contaminants present in the dataset exists, independent of other environmental variables.

Further, Distance-based linear modeling (DistLM) was used to assess the relative importance of (1) environmental factors (sediment texture, temperature, oxygen, and TOC) in shaping the distribution of contaminants (concentrations and indices) and (2) environmental factors and contaminants in explaining the observed reproductive responses in amphipods. DistLM is a multivariate linear model that uses explanatory information provided as a distance matrix to generate the most parsimonious combination of predictors to explain variation in the response variables while accounting for the potential overlap of the predictors. In DistLM, pseudo-F statistic for testing the general null hypothesis of no relationship is used and p values for individual predictors are obtained through permutations (in this study 9999). Thus, the approach is robust to non-normal data, and errors do not need to be normally distributed (McArdle & Anderson, 2001).

To avoid collinearity between predictors, variables that were strongly correlated with each other ($r > 0.8$; Table S5) were omitted, and no interactions were considered due to the relatively low number of observations. The subsets of variables were established using forward selection based on the multivariate analogue to the small-sample-size corrected version of the Akaike Information Criterion (AICc). Relationships between environmental parameters and reproductive attributes were initially examined by analyzing each predictor separately (marginal tests). Then, partial regressions were used to characterize the relationships accounting for the effect of the remaining variables by sequential tests with step-wise selection procedures and AICc as the selection criterion. The models were visualized using a distance-based Redundancy Analysis plot (dbRDA).

3. Results

3.1. Environmental data

The bottom depth ranged from 12 to 134 m in BoS, 19 to 42 m in WGB, 16 to 52 m in GoF, and 24 to 38 m in GoR. TOC in sediments ranged from 0.3 to 5.9 % in GoF, 0.5–1.1 % in GoR, 0.4–3.8 % in BoS, and 0.7–2.3 % in WGB. The other environmental parameters, including salinity (1.4–7.2), near-bottom temperature (2.1–5.7℃), oxygen concentration (6.5–12.5 mg l^{-1}), and sediment texture (percentage of clay, silt, and sand) were highly variable across the subbasins (Table S1). The salinity was the main parameter associated with the variability between the sites and the only parameter that displayed a statistically significant difference ($p < 0.05$) between the subbasins (Fig. S1). Compared to other subbasins, lower salinity, and higher temperature were recorded for GoF, with the opposite pattern for WGB (Fig. S1; Table S1). Silt prevailed in the GoF and BoS sediments, 59 % and 56 %, respectively, whereas sand dominated in the GoR and the WGB sediments (Fig. 2; Table S1). WGB and BoS sites generally displayed higher salinity and more sandy sediments. In addition, GoF and GoR sites were characterized by better oxygenation (Fig. 2), although GoF sites were more variable in terms of the sediment grain size and oxygenation, whereas GoR sites had more uniform oxygenation and sandy sediments (Fig. 2). This variability in GoF was mostly related to the sites in the Neva Estuary, the eastern part of GoF, that had lower salinity and higher proportion of silt in the sediment (Table S1).

3.2. Contaminant data

The highest variability in the contaminant levels was observed for BoS, mostly due to the highly contaminated sites SU57 and SU58 (Sundsvall), and GoF (near-harbour areas in the Neva Estuary) subbasins. The BoS sediments had the highest concentrations of metals (mean 283 mg kg^{-1} DW with a PLI value of 13.8), PAHs (mean 30.3 μg g^{-1} DW), and PCBs (mean 23.7 ng g^{-1}DW; Table S2). The GoF sediments had the highest BT loading (mean 61.7 ng g^{-1}DW), but also high variability of the PCBs, Pb, and PLI values (Fig. S2).

The total metal concentration in sediments was 37 – 364 mg kg^{-1} DW, with the lowest and the highest values at the G3 site (GoR) and Bråviken 8 (WGB), respectively. Moreover, high As levels were recorded at SU57 and SU58 sites (BoS) and high Cd in the Neva Estuary at 3F, 4F, and 9F sites (GoF; Fig. 3). The total PAH concentrations ranged from non-detectable levels (G3 site, GoR) to 81 μg g^{-1} DW (SU58 site, BoS; Table S2). The total PCB concentations ranged from non-detectable levels observed at several sites in different subbasins to 81 ng g^{-1} DW (SU58 site, BoS). Finally, the total BT concentrations were frequently non-detectable, but relatively high in GoF, sites 2F, 3F, 4F, 9F and 2ug (up to 335 ng g^{-1} DW; Table S2).

3.3. Associations between environmental parameters and contaminants

The environmental parameters explained 90.1 % of the fitted and 52.5 % of the total variation in the contaminant load across the stations (DistLM; Table 1, Table S5, Fig. 4). Based on the environmental variability and contaminants, the sampling sites formed two groups, where the first consisted of the stations located in the Neva Estuary in GoF, which are characterized as stations with high TOC, BTs and low PCBs concentrations, and the second comprised the remaining sites, with higher PCB concentrations than the first group. Variability in some GoF stations was influenced by salinity and/or temperature, while higher clay content in sediments played a significant role in the differences in chemical load observed in the majority of other stations (Fig. 4). Overall, higher levels of contaminants were found at lower temperatures, higher salinities and in fine-grain sediments.

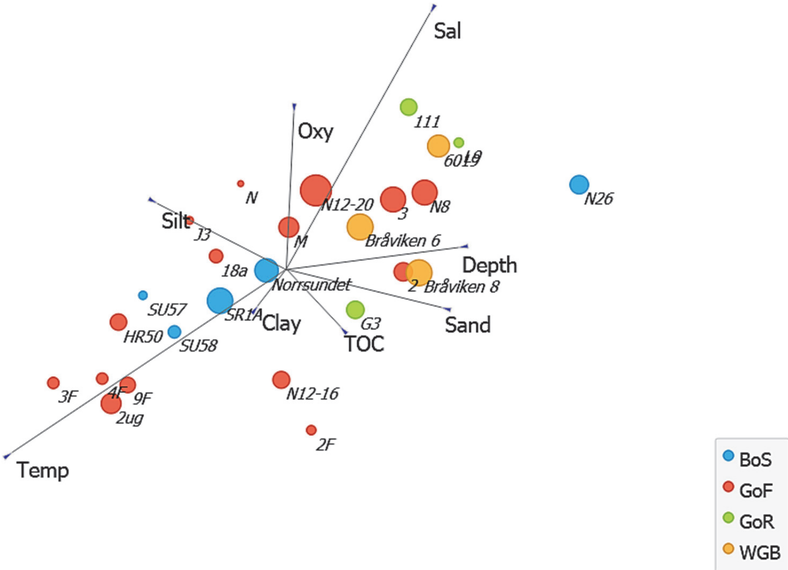

Fig. 2. Association between the environmental variables and stations across the subbasins. The vectors show the variable variability (length) and orientation; the bubble size corresponds to the number of amphipod females collected for the analysis from each station; the subbasins are color-coded according to the legend. The station names are indicated on the circles, and arrows carry the environmental variable names; please see Table S1 for the complete list of the stations and environmental variables.

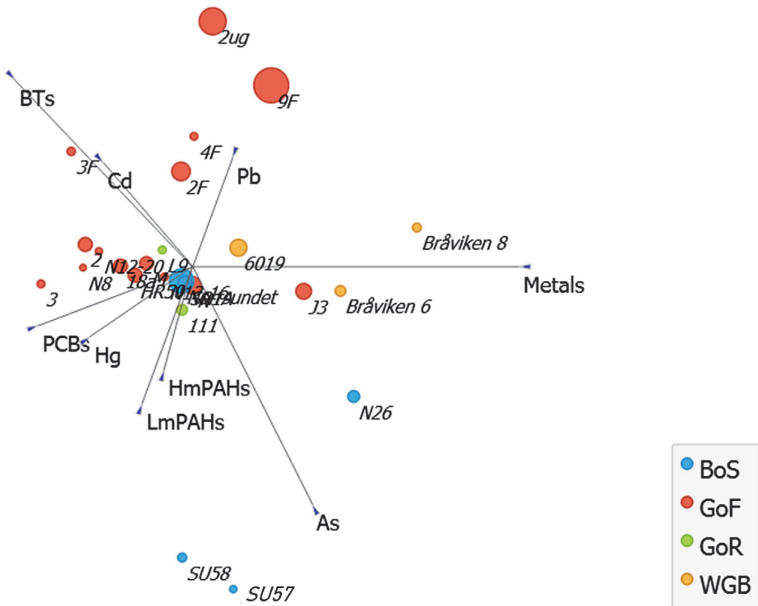

Fig. 3. Associations between the contaminants and stations across the subbasins. The vectors show the chemical contaminant variability (length) and orientation; see Table S2 for the complete list. The circle size corresponds to the sediment TOC value at the station, with the color coding indicating the subbasin. The circles are labeled with station names as in Fig. 2.

3.4. Biological parameters

Due to natural phenological variability across the subbasins and differences in the time of mating (December – January), the embryos used for analysis were in different developmental stages, with the average stage ranging from ~3 to 7, with the least developed embryos found in GoF (Table S4; Fig. 5). Fecundity between stations also varied (13.6–42.2 eggs female^{-1}), and the ranges for the proportion of the

Table 1
DistLM output for marginal and sequential tests linking environmental factors to the main contaminant groups at the sampling stations. The best model was selected based on Akaike information criteria (AICc) for all sites. Pseudo-*F* value, a multivariate analogue of F-value for linear regression; Adj. R^2, adjusted proportion of explained variation attributable to variables in a model; P, probability; R^2, the proportion of explained variation attributable to each variable; R^2 (cum), the cumulative proportion of variation; rs.df, residual degrees of freedom.

Marginal tests				
Variables	SS (trace)	Pseudo-*F*	P	R^2
Clay/silt	21.79	6.686	0.004	0.218
Temperature	9.936	2.648	0.06	0.099
Salinity	18.71	5.524	0.005	0.187
TOC	4.617	1.646	0.17	0.064
Oxygen	8.866	2.335	0.08	0.089
Depth	2.27	0.557	0.64	0.023
Sand	16.919	4.888	0.009	0.169
Sequential tests				
Variables	P	R^2	R^2 cum	rs.df
Clay/silt	0.001	0.18	0.2179	24
Temperature	0.009	0.32	0.37713	23
Salinity	0.02	0.39	0.46281	22
TOC	0.03	0.45	0.54072	21
Oxygen	0.12	0.48	0.58348	20
Overall best solution				
Adj. R^2	R^2	RSS	No. Vars	Selections
0.479	0.583	41.652	5	2–6

aberrant embryos (%AbEmb) and females carrying more than 1 aberrant embryo (%Fem > 1) were 2–22 % and 1–80 %, respectively. The lowest mean %AbEmb and %Fem > 1 values were recorded in GoR (6 % and 33 %, respectively) and the highest in BoS (12 and 56 %; Table S4).

Ordination of the reproductive variables (Fecundity, Stage, %AbEmb and %Fem > 1) revealed substantial variability between the subbasins, with a significant correlation between %AbEmb and %Fem > 1 (Spearman *rho*: 0.6, $p < 0.004$; Fig. 5). The highest variability for the reproductive variables was detected in GoF and the lowest in WGB (Fig. S3). Most of the GoF sites were characterized by relatively high levels of both %Fem > 1 and %AbEmb, albeit a lower proportion of aberrant embryos was recorded in the Neva Estuary.

3.5. Linking reproductive variables to environmental factors and contaminants

The classification of the sampling sites by cluster analysis revealed four clusters differing by the loads and compositions of contaminants (Fig. 6). Cluster 1 consists of only two highly contaminated sites SU57 and SU58 (Sundsvall, BoS), with very high levels of metals (As, Hg, Cu, and Ni), PAHs and PCBs. Cluster 2 consists of five sites located in GoF and having relatively high levels of BTs and some metals (Cd and Pb), whereas the other two clusters combine sites located in multiple subbasins and characterized by diverse mixtures of PAHs, PCBs, and metals. Cluster 3 includes sites with low levels of metals and intermediate levels of PAHs, and Cluster 4 includes sites with consistently high levels of PCBs, and intermediate-to-high levels of some PAHs and metals (Cr and Ni). Thus, none of the clusters represented non-polluted conditions. When %Fem > 1 was compared across the four clusters, significantly higher values were found for Cluster 4 compared to Clusters 3 (Unpaired t-Test: t-value = −2.27, df = 17, $p < 0.037$; Fig. 6) and to Cluster 2 (t-value = −2.52, df = 14; $p < 0.025$). For %AbEmb, the differences between the clusters were not significant, albeit higher values were observed for C4 compared to C3 and C2 (0.11 vs 0.082 and 0.077, respectively).

When both chemical and non-chemical predictors were considered, reproductive aberrations were significantly associated with environmental factors (salinity, temperature, TOC, and proportion of clay), PAH concentrations (naphthalene; NAP and acenaphthene; DBAHA), and PLI as a proxy for metals (DistLM; Table 2, Fig. 7). The individual

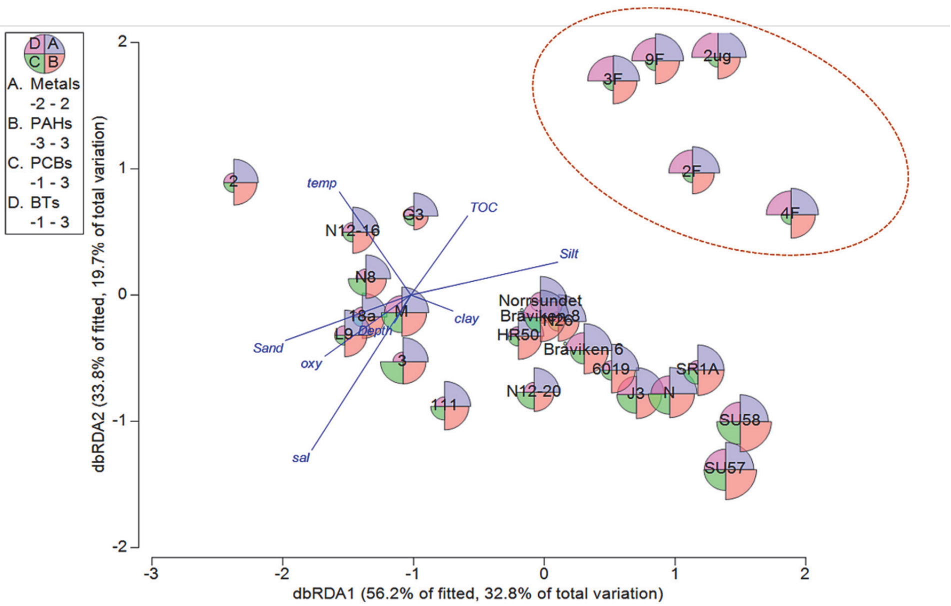

Fig. 4. dbRDA biplot for DistLM relating chemical load (by contaminant group, metals, PAHs, PCBs, and BTs) in sediment as multivariate response variables to non-chemical predictors: depth, temperature, oxygen, TOC, and sediment composition (clay, silt, and sand). The orange ellipse indicates stations located in the Neva Estuary (GoF) and characterized by high TOC, BTs and low PCBs concentrations.

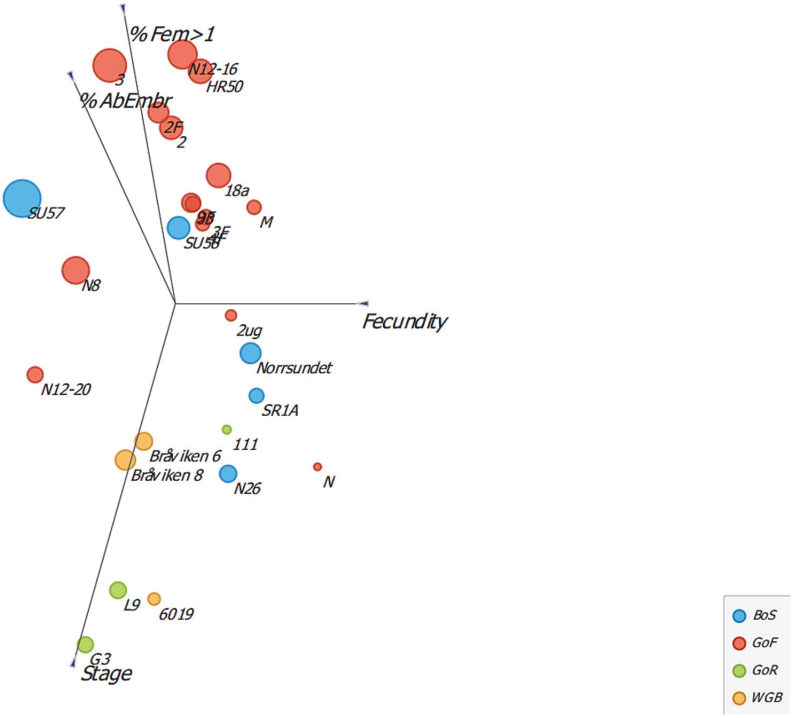

Fig. 5. Associations between the reproductive variables (fecundity, %AbEmb, %Fem > 1, and stage) and stations across the subbasins. The vectors show the variable variability (length) and orientation; the bubble size corresponds to the number of individuals analyzed from each station, and the color code indicates the subbasin. Red color represents GoF, blue: BoS, green: GoR and yellow: WGB.

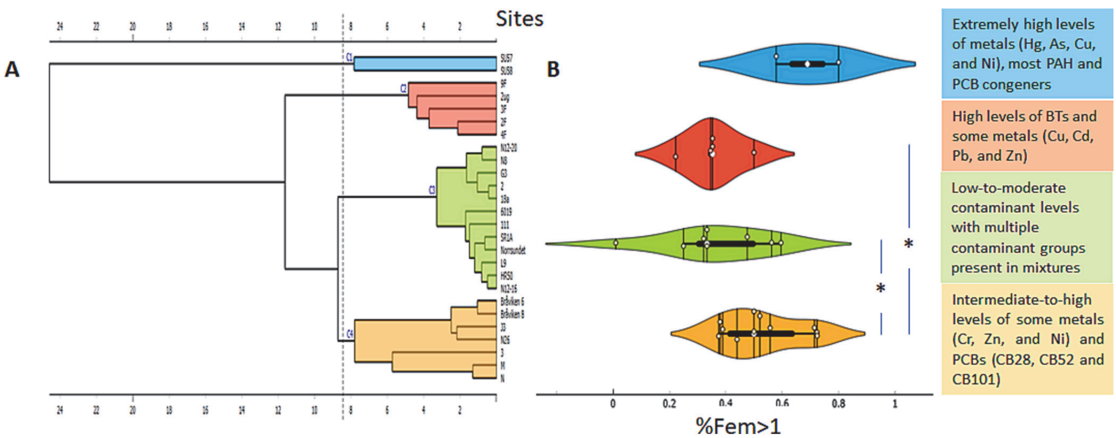

Fig. 6. Variability of the reproductive aberrations across sites grouped according to their contaminant load. **(A)** Hierarchical clustering of the sampling sites according to the contaminant load (Ward linkage), yielding four clusters (C1 to C4), and **(B)** Violin plots for the proportion of the females carrying more than 1 aberrant embryo (%Fem > 1). The width of each curve corresponds with the approximate frequency of data points in each region. The densities are additionally annotated with the median value and interquartile range, shown as a black boxplot within each violin plot. Significant differences between the groups detected by the unpaired *t*-test are indicated by an asterisk ($p < 0.05$). Note that C1 was not included in the statistical comparison because it was comprised of only 2 stations.

contributions of the environmental variables ranged from 14 % (silt) to 34 % (salinity) of the total variance explained, whereas contaminants contributed from 13 % (NAP) to 18 % (PLI) (Table 2). The first two axes of the dbRDA plot of reproductive aberrations explained 80 % of the total and 99 % of the fitted variation, indicating that most of the salient patterns in the fitted model were captured (Fig. 7). Based on the model, the aberration frequencies increased with the contaminant concentrations, temperature, and salinity and decreased in organic-rich sediments

Table 2
DistLM output for marginal and sequential tests showing relationships between environmental factors, chemical variables, and reproductive aberrations in *Monoporeia affinis*.

Variable			Variable				
Marginal tests	P	R^2	Sequential tests	P	R^2	R^2 cum	rs.df
Physical properties							
(−) depth	0.945	<0.01					
(+) sal	**0.001**	**0.34**	sal	0.001	0.33957	0.33957	23
(−) oxy	0.188	0.07					
(+) temp	**0.001**	**0.33**	temp	0.001	0.17665	0.51622	22
(−) TOC	**0.004**	**0.20**	TOC	0.007	0.11002	0.62624	21
(−) clay	0.191	0.07	clay	0.036	0.045144	0.80329	18
(−) silt	**0.029**	**0.14**					
Contaminants							
(+) PLI	**0.042**	**0.18**					
(+) As	0.615	0.02					
(+) Cu	0.930	<0.01					
(+) Hg	0.180	0.07	Hg	0.045	0.0599	0.68614	20
(−) Ni	0.701	0.02					
(+) PAHs	0.218	0.07					
(+) NAP	**0.028**	**0.13**					
(+) DBAHA	**0.031**	**0.14**					
(+) FLE	0.124	0.09					
(+) PCBs	0.19	0.07					
(+) PCB-28	0.458	0.03					
(+) PCB-118	0.241	0.06					
(+) PCB-180	0.168	0.08	PCB-180	0.019	0.072004	0.75815	19

P, probability; R^2, the proportion of explained variation attributable to each variable; R^2 (cum), the cumulative proportion of variation; rs.df, residual degrees of freedom; (+/−), positive/negative relationship with %AbEmb and %Fem > 1.

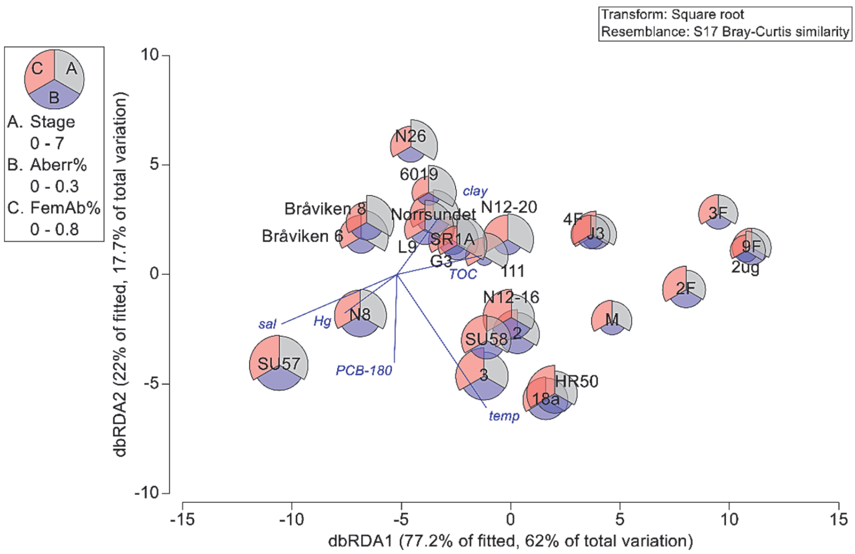

Fig. 7. Constrained ordination (dbRDA biplot) of the fitted values of the most parsimonious DistLM for reproductive aberration frequencies in amphipods (%AbEmb and %Fem > 1) as a multivariate response to the predictors identified by the model: contaminants (PCB-180 and Hg) and non-chemical environmental factors (temperature, TOC, salinity, and clay). In addition to the aberration frequencies, a developmental stage of the embryos is also shown.

with a high proportion of fine (clay and silt) particles.

There was a significant increase of aberrations with increased contaminant concentrations, i.e., metals (PLI, Hg), PAHs (NAP and DBAHA), and PCBs (PCB180). Given the high levels of cross-correlations between different metals and PLI values as well as across congeners of PAHs and PCBs, those were excluded from the list of candidate predictors in DistLM to avoid multicollinearity. Thus, it is likely that the detected effects are not specific to these compounds but reflected toxicity exerted by other congeners in the contaminant mixture. Therefore, the percentage of variance explained by a single component of a mixture of covarying compounds does not necessarily reflect the contribution of the overall chemical exposure to the observed effects.

4. Discussion

Using a geographically broad dataset on embryo aberrations in common amphipod *Monoporeia affinis*, environmental factors and contaminant levels in sediment, we found that aberration frequency is driven by both environmental factors (salinity, temperature, TOC and silt proportion) and contaminants (metals [PLI and Hg], PAHs [NAP and DBAHA] and PCBs [PCB180]). These findings confirm earlier reports that embryo aberrations in this species respond to chemical pollution (Sundelin et al., 2008a, Löf et al., 2016a; Löf et al., 2016b), and that this response is detectable *in situ*. Both components of the ReproIND indicator (i.e., percentage of aberrant embryos in a population and percentage of females carrying more than 1 aberrant embryo) increased at higher contaminant load (Fig. 7). Therefore, the proposed indicator demonstrates its applicability in all the investigated Baltic Sea subbasins for assessing contaminant impacts, even in the face of high environmental variability and substantial differences in contaminant load across the study areas.

4.1. Environmental variability and contaminants

The differences between the amphipod habitats in the western (BoS and WGB) and the eastern (GoR and GoF) subbasins were substantial, both in relation to the environmental parameters (Fig. S1) and the contaminant load (Fig. S2), with no noticeable differences in the TOC values. The eastern areas were characterized by significantly higher temperatures, better oxygenation, and mostly fine-grain sediments, albeit the grain size distribution varied across the GoF sites, with the finest sediment in the Neva Estuary, coinciding with the lowest salinity. Conversely, higher salinities and often more sandy sediments were common for the western sites.

The elevated contaminant levels were associated with high salinity, low temperature, and fine-grained sediments, consistent with other reports (Höglund and Jonsson, 2008, Löf et al., 2016a; Löf et al., 2016b, Erm et al., 2021). These observations were primarily driven by the sites with high contaminant loads in WGB and BoS and relatively low levels of all contaminants in GoR. Among the contaminant groups, trace metals were the most widespread contaminants in all subbasins, with particularly high concentrations of As in the Bråviken Bay (WGB) and Hg in the Bothnian Sea, whereas extremely high PAH and PCB levels were recorded in Sundsvall (SU57 and SU58; BoS). The latter is related to the historical contamination by pulp and paper mills leading to elevated levels of metals and organic pollutants in the sediments, along with high fiber concentrations (Höglund and Jonsson, 2008; Apler et al., 2014, 2019). Notably, the lowest contaminant levels were consistently found in the GoR sediments, with BTs being common in the Neva Estuary, particularly, in the proximity to shipping lanes (e.g., sites 3F, 4F, 9F, and 2ug; GoF).

4.2. Linkage between the embryo aberrations and contaminants

The environmental factors accounting for the differences between the subbasins explained most of the variability in the embryo aberrations, which was expected, whereas individual contaminants explained up to 18 % of the captured variability. Both parameters describing reproductive aberration frequency in a population (%AbEmb and %Fem > 1) were positively correlated with salinity and temperature and negatively with TOC and silt. An increase in embryo aberrations at higher temperatures agrees with the current knowledge on *M. affinis* ecology (Wiklund & Sundelin, 2001, Eriksson Wiklund and Sundelin, 2004). Furthermore, similar negative effects of temperature on embryo development have been observed in other amphipod species in the region (Berezina et al., 2017).

In the Neva Estuary, aberration frequency was relatively low, despite very high BT concentrations (on a global scale; Meador et al., 1997), coinciding with high TOC levels and clay/silt in the sediments. High organic carbon content and fine-grained sediments have been reported to convey reduced contaminant bioavailability (Kreitinger et al., 2007; Baran et al., 2019). As hydrophobic BTs are sorbed to organic matter, their bioavailability is reduced (Rüdel, 2003, Cornelissen et al., 2005), leading to limited uptake and bioaccumulation as reported for tributyltin in amphipod *Rhepoxynius abronius* inhabiting high-TOC sediments (Meador et al., 1993,1997). Further, interspecific variability in bioaccumulation and capacity to metabolize contaminants, such as TBT, as well as differences in the depth and rate of feeding have been reported in amphipods (Byrén et al., 2002, Ohji et al., 2002). A further study investigating intraspecies variability in *M. affinis*, might offer an explanation for the low embryo aberration rates from the Neva Estuary.

Among the analysed contaminants, the metals (PLI, Hg), PAHs (NAP and DBAHA), and PCBs (PCB180 but also PCB28 and PCB118; Table 2) explained the most variability in the reproductive aberrations (% AbEmb, %Fem > 1). These findings corroborate those by Löf et al., (2016a) from two localities in the Gulf of Bothnia, where the %AbEmb variability was best explained by PCBs (with PCB180 being the primary driver), PAHs (Phenanthrene, 1-Methylphenanthrene, benzo[ghi]perylene) and Cd. A higher aberration rate near known pollution sources has been reported as a general tendency in *M. affinis* and other amphipod species (Sundelin & Eriksson, 1998, Bach et al., 2010, Reutgard et al., 2014, Tairova and Strand, 2022). However, as contaminants occur in mixtures and under heterogeneous conditions, the univariate relationships between specific pollutants and reproductive responses should not be expected, which further motivates the development of biological indicators of contaminant exposure and effects applicable across ecosystems.

The significant contribution of contaminants to the embryo aberration variability reported here supports the applicability of the ReproIND indicator in all Baltic Sea areas where *M. affinis* and several other amphipod species are common. Previous studies exploring environmental and contaminant drivers on the reproductive aberrations in *M. affinis* were limited to the Gulf of Bothnia (Reutgard et al., 2014, Löf et al., 2016a; Löf et al., 2016b). Despite extensive studies on contaminants and their biological effects in the Baltic Sea region (Kankaanpää et al., 2022), the use of biological effect indicators in monitoring programs is currently limited. One reason for this is a lack of standardization efforts and evaluations based on data from different areas and populations existing under contrasting conditions, especially concerning the temperature and salinity gradients known to shape the physiology and phenology of the Baltic biota (Eriksson Wiklund and Sundelin, 2004, Nohrén et al., 2009, Larsson et al., 2017). Therefore, our findings provide a strong background for the future development of the ReproIND indicator, particularly for setting regional thresholds to account for the confounding reproductive responses to the temperature, salinity, TOC, and sediment grain size distribution in different subbasins and sampling locations.

Moreover, to expand the biological effect assessment and include non-reproductive responses, ReproIND can be combined with other biomarkers, e.g., antioxidant defense, geno- and cytotoxicity (Turja et al., 2020), metabolic activity, oxidative balance, neurotoxicity (Löf et al., 2016a; Löf et al., 2016b), and DNA adductome (Gorokhova et al., 2020). An integrated approach employing a battery of biological effects would provide a better understanding of the broad impact of multiple mixed effects of contaminants (Lehtonen et al., 2014,2016, Turja et al., 2014a, Berezina et al., 2019) and support monitoring and management of environmental contaminants at the national, regional, and local levels.

5. Conclusion

Using data on reproductive aberrations in *M. affinis*, chemical contaminants and environmental factors from four subbasins of the Baltic Sea, we found that both environmental factors and contaminants contributed significantly to the variability in the embryo aberrations. The most influential natural drivers associated with high aberration frequency were sandy sediments, high temperature and salinity, and low TOC. Among the chemical contaminants, the significant contributors were metal mixtures (assayed as Tomlinson Pollution Level Index for metals) and total mercury concentrations, low-molecular PAHs (NAP and DBAHA) and high-molecular PCBs (PCB-180). However, high cross-correlations for PAHs and PCBs imply that other exposure metrics, such as chemical activity (Gobas et al., 2018) should be explored as a dose metric to assess the mixture effects of these hydrophobic substances. Nevertheless, our findings justify the application of reproductive aberrations as a biological effect indicator in the Baltic Sea, and, perhaps, other areas where pollution levels are of concern.

CRediT authorship contribution statement

N. Kolesova: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **S. Sildever:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition. **E. Strode:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Data curation. **N. Berezina:** Writing – review & editing, Funding acquisition, Data curation. **B. Sundelin:** Writing – review & editing, Methodology, Data curation. **I. Lips:** Writing – review & editing, Funding acquisition. **I. Kuprijanov:** Writing – review & editing, Funding acquisition, Data curation. **F. Buschmann:** Writing – review & editing, Data curation. **E. Gorokhova:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data has been made available as supplementary material.

Acknowledgments

The crew of r/v Salme is thanked for their assistance during the sampling campaigns. Oliver Samlas and Ieva Barda are thanked for assisting in sampling M. affinis and sediment collection. This study was supported by funding from Environmental Investment Centre (KIK 10313, 16300, 17253, RE.4.07.22-0014) [NK, IK], Estonia-Russia Cross-Border Cooperation Program 2014-2020 (HAZLESS project ER90) [IL, IK, NK, NB], European Regional Development Fund Postdoctoral Research Grant 1.1.1.2/16/l/001 (application No 1.1.1.2/VIAA/3/19/465) [ES], Swedish National Marine Monitoring Program (SNMMP) [BS, EG], Interreg Baltic Sea Region co-funded by the European Union (#S007 10/2022-10/2024) [NK, IK, EG, BS, ES], Estonian Research Council grant (PSG735) [SS], European Biodiversity Partnership Biodiversa + (D2P, Proposal number: 2021-473) [IK, NK, EG, BS, ES].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2024.111837.

References

Anderson, M., Gorley, R.N., Clarke, K., 2008. PERMANOVA+ for primer: Guide to software and statistical methods.
Anderson, 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Wiley StatsRef Stat Ref Online:1–15.
Apler, A., Nyberg, J., Jönsson, K., Hedlund, I., Heinemo, S.-Å., Kjellin, B., 2014. Mapping of fiber in the sediments along the Västernorrland coast. Geological Survey of Sweden. [in Swedish] http://resource.sgu.se/produkter/sgurapp/s1416-1-rapport.pdf [accessed 07.07.2023].
Apler, A., Snowball, I., Frogner-Kockum, P., Josefsson, S., 2019. Distribution and dispersal of metals in contaminated fibrous sediments of industrial origin. Chemosphere 215, 470–481.
Bach, L., Fischer, A., Strand, J., 2010. Local anthropogenic contamination affects the fecundity and reproductive success of an Arctic amphipod. Mar. Ecol. Prog. Ser. 419, 121–128.
Baran, A., Mierzwa-Hersztek, M., Gondek, K., Tarnawski, M., Szara, M., Gorczyca, O., Koniarz, T., 2019. The influence of the quantity and quality of sediment organic matter on the potential mobility and toxicity of trace elements in bottom sediment. Environ. Geochem. Health 41, 2893–2910.
Berezina, N.A., Gubelit, Y.I., Polyak, Y.M., Sharov, A.N., Kudryavtseva, V.A., Lubimtsev, V.A., Petukhov, V.A., Shigaeva, T.D., 2017. An integrated approach to the assessment of the eastern gulf of Finland health: a case study of coastal habitats. J. Mar. Syst. 171, 159–171.
Berezina, N.A., Lehtonen, K.K., Ahvo, A., 2019. Coupled application of antioxidant defense response and embryo development in amphipod crustaceans in the assessment of sediment toxicity. Environ. Toxicol. Chem. 38, 2020–2031.
Berezina, N.A., Sharov, A.N., Chernova, E.N., Malysheva, O.A., 2022. Effects of diclofenac on the reproductive health, respiratory rate, cardiac activity, and heat tolerance of aquatic animals. Environ. Toxicol. Chem 41, 677–686.
Blomqvist, S., Lundgren, L., 1996. A benthic sled for sampling soft bottoms. Helgoländer Meeresunters. 50, 453–456.
Byrén, L., Ejdung, G., Elmgren, R., 2002. Comparing rate and depth of feeding in benthic deposit-feeders: a test on two amphipods, Monoporeia affinis (lindström) and Pontoporeia femorata kröyer. J. Exp. Mar. Bio. Ecol. 281, 109–121.
Cornelissen, G., Gustafsson, Ö., Bucheli, T.D., Jonker, M.T.O., Koelmans, A.A., Van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environ. Sci. Technol. 39, 6881–6895.
Cornelissen, G., Pettersen, A., Nesse, E., Eek, E., Helland, A., Breedveld, G.D., 2008. The contribution of urban runoff to organic contaminant levels in harbour sediments near two norwegian cities. Mar. Pollut. Bull. 56, 565–573.
Dempster, A.P., Laird, N.M., Rubin, D.B., 1977. Maximum likelihood from incomplete data via the EM algorithm. J. R. Stat. Soc. Ser. B 39, 1–22.
Demšar, J., Leban, G., Zupan, B., 2007. FreeViz-an intelligent multivariate visualization approach to explorative analysis of biomedical data. J. Biomed. Inform. 40, 661–671.
Eriksson, A.K., Sundelin, B., Broman, N. C., 1996. Effects on Monoporeia affinis of HPLC-fractionated extracts of bottom sediments from a pulp mill recipient. St Lucie Press, Florida.
Eriksson Wiklund and Sundelin, 2004. Biomarker sensitivity to temperature and hypoxia – a seven year field study. Mar. Ecol. Prog. Ser. 274, 209–214.
Erm, A., Buschmann, F., Aan, A., 2021. Prioriteetsete ainete ja toiteainete vertikaalsest jaotusest Väinameres ja Liivi lahes. Tallinna Tehnikaülikooli Meresüsteemide instituut. KIK nr 16300.
European Commission, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive).
Gobas, F.A.P.C, Mayer, P., Parkerton, T.F., Burgess, R.M., van de Meent, D., Gouin, T., 2018. A chemical activity approach to exposure and risk assessment of chemicals. EPA Public Access. Environ. Toxicol. Chem. 37, 1235–1251.
Gorokhova, E., Löf, M., Halldórsson, H.P., Tjärnlund, U., Lindström, M., Elfwing, T., Sundelin, B., 2010. Single and combined effects of hypoxia and contaminated sediments on the amphipod Monoporeia affinis in laboratory toxicity bioassays based on multiple biomarkers. Aquat. Toxicol. 99, 263–274.
Gorokhova, E., Martella, G., Motwani, N.H., Tretyakova, N.Y., Sundelin, B., Motwani, H. V., 2020. DNA epigenetic marks are linked to embryo aberrations in amphipods. Sci. Rep. 10, 1–11.
Güler, C., Thyne, G.D., McCray, J.E., Turner, K.A., 2002. Evaluation of graphical and multivariate statistical methods for classification of water chemistry data. Hydrogeol. J. 10, 455–474.
HELCOM, 2010. Ecosystem health of the Baltic Sea 2003-2007: HELCOM initial holistic assessment. Balt Sea Environ. Proc. 122.
Havs- och vattenmyndigheten, 2018. Metals and environmental toxins - base and indicative values for sediments for effect-based assesment. The Sea and Water Authority report nr. 31. [in Swedish].
HELCOM, 2018a. Inputs of hazardous substances to the Baltic Sea. Balt. Sea Environ. Proc. 161.
HELCOM, 2018b. Reproductive disorders: malformed embryos of amphipods. HELCOM supplementary indicator report.
HELCOM, 2018c. State of the Baltic Sea- Second HELCOM holistic assessment, 2011-2016. Balt Sea Environ Proc 155:4–7. https://helcom.fi/wp-content/uploads/2019/06/BSEP155.pdf [accessed 07.07.2023].
HELCOM, 2021. HELCOM Baltic Sea Action Plan - 2021 update.
HELCOM ACTION, 2021. Conditions that influence Good Environmental Status (GES) in the Baltic Sea.
HELCOM, 2023. HELCOM Thematic assessment of hazardous substances, marine litter, underwater noise and non-indigenous species 2016-2021. Balt. Sea Environ Proceeding 190.
Höglund, L.O. and Jonsson, K., 2008. Utredning rörande kvicksilvertunnor i Sundsvallsbukten. Kemakata Konsult AB.Dnr 577-8179-07.
Jędruch, A., Kwasigroch, U., Bełdowska, M., Kuliński, K., 2017. Mercury in suspended matter of the Gulf of Gdańsk: origin, distribution and transport at the land–sea interface. Mar. Pollut. Bull. 118, 354–367.

Kankaanpää, H.T., Turja, R., Lehtonen, K.K., 2.22) Advanced monitoring of harmful substances and their effects in the Baltic Sea is desired: A comment on Kanwischer et al. (2021). Ambio 51:1611–1613.
Kholodkevich, S.V., Kuznetsova, T.V., Sharov, A.N., Kurakin, A.S., Lips, U., Kolesova, N., Lehtonen, K.K., 2017. Applicability of a bioelectronic cardiac monitoring system for the detection of biological effects of pollution in bioindicator species in the Gulf of Finland. J. Mar. Syst. 171, 151–158.
Kreitinger, J.P., Neuhauser, E.F., Doherty, F.G., Hawthorne, S.B., 2007. Greately reduced bioavailability and toxicity of polycyclic aromatic hydrocarbons to *Hyalella azteca* in sediments from manufactured-gas plant sites. Environ. Toxicol. Chem. 26, 1146–1157.
Kuprijanov, I., Väli, G., Sharov, A., Berezina, N., Liblik, T., Lips, U., Kolesova, N., Maanio, J., Junttila, V., Lips, I., 2.21) Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland. Mar Pollut Bull 170.
Lam, P.K.S., Gray, J.S., 2003. The use of biomarkers in environmental monitoring programmes. Mar. Pollut. Bull. 46, 182–186.
Larsson, J., Lind, E.E., Corell, H., Grahn, M., Smolarz, K., Lönn, M., 2017. Regional genetic differentiation in the blue mussel from the Baltic Sea area. Estuar. Coast Shelf Sci. 195, 98–109.
Lehtonen, K.K., Sundelin, B., Lang, T., Strand, J., 2014. Development of tools for integrated monitoring and assessment of hazardous substances and their biological effects in the Baltic Sea. Ambio 43, 69–81.
Lehtonen, K.K., Turja, R., Budzinski, H., Devier, M.H., 2016. An integrated chemical-biological study using caged mussels (*Mytilus trossulus*) along a pollution gradient in the Archipelago Sea (SW Finland, Baltic Sea). Mar. Environ. Res. 119, 207–221.
Li, Z., Zhang, W., Shan, B., 2022. Effects of organic matter on polycyclic aromatic hydrocarbons in riverine sediments affected by human activities. Sci. Total Environ. 815, 152570.
Löf, M., Sundelin, B., Bandh, C., Gorokhova, E., 2016a. Embryo aberrations in the amphipod Monoporeia affinis as indicators of toxic pollutants in sediments: a field evaluation. Ecol. Indic. 60, 18–30.
Löf, M., Sundelin, B., Liewenborg, B., Bandh, C., Broeg, K., Schatz, S., Gorokhova, E., 2016b. Biomarker-enhanced assessment of reproductive disorders in Monoporeia affinis exposed to contaminated sediment in the Baltic Sea. Ecol .Indic. 63, 187–195.
Lyons, B.P., Bignell, J.P., Stentiford, G.D., Bolam, T.P.C., Rumney, H.S., Bersuder, P., Barber, J.L., Askem, C.E., Nicolaus, M.E.E., Maes, T., 2017. Determining good environmental status under the marine strategy framework directive: case study for descriptor 8 (chemical contaminants). Mar. Environ. Res. 124, 118–129.
Martín-Díaz, M.L., Blasco, J., Sales, D., DelValls, T.A., 2004. Biomarkers as tools to assess sediment quality: laboratory and field surveys. TrAC Trends Anal. Chem. 23, 807–818.
McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82, 290–297.
McCarty, L.S., Mackay, D., 1993. Enhancing ecotoxicological modeling and assessment. Environ. Sci. Technol. 27, 1719–1728.
Meador, J.P., Varanasi, U., Krone, C.A., 1993. Differential sensitivity of marine infaunal amphipods to tributyltin. Mar. Biol. Int. J. Life Ocean Coast Waters 116, 231–239.
Meador, J.P., Krone, C.A., Wayne Dyer, D., Varanasi, U., 1997. Toxicity of sediment-associated tributyltin to infaunal invertebrates: species comparison and the role of organic carbon. Mar. Environ. Res. 43, 219–241.
Nohrén, E., Pihl, L., Wennhage, H., 2009. Spatial patterns in community structure of motile epibenthic fauna in coastal habitats along the Skagerrak - Baltic salinity gradient. Estuar. Coast Shelf Sci. 84, 1–10.
Ohji, M., Takeuchi, I., Takahashi, S., Tanabe, S., Miyazaki, N., 2002. Differences in the acute toxicities of tributyltin between the caprellidea and the gammaridea (crustacea: amphipoda). Mar. Pollut. Bull. 44, 16–24.
Pérez, E., Hoang, T.C., 2017. Chronic toxicity of binary-metal mixtures of cadmium and zinc to Daphnia magna. Environ. Toxicol. Chem. 36, 2739–2749.
Podlesińska, W., Dąbrowska, H., 2019. Amphipods in estuarine and marine quality assessment – a review. Oceanologia 61, 179–196.
Queirós, A.M., Strong, J.A., Mazik, K., Carstensen, J., Bruun, J., Somerfield, P.J., Bruhn, A., Ciavatta, S., Flo, E., Bizsel, N., Özaydinli, M., Chuševe, R., Muxika, I., Nygård, H., Papadopoulou, N., Pantazi, M., Krause-Jensen, D., 2016. An objective framework to test the quality of candidate indicators of good environmental status. Front. Mar. Sci. 3.
R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing.
Raymond, C., Gorokhova, E., Karlson, A.M.L., 2021. Polycyclic aromatic hydrocarbons have adverse effects on benthic communities in the Baltic Sea: implications for environmental status assessment. Front. Environ. Sci. 9, 1–12.
Reutgard, M., Eriksson Wiklund, A.K., Breitholtz, M., Sundelin, B., 2014. Embryo development of the benthic amphipod Monoporeia affinis as a tool for monitoring and assessment of biological effects of contaminants in the field: a meta-analysis. Ecol. Indic. 36, 483–490.
Rüdel, 2003. Case study: bioavailability of tin and tin compounds. Ecotoxicol. Environ. Saf. 56, 180–189.
Schneider, B., Ceburnis, D., Marks, R., Munthe, J., Petersen, G., Sofiev, M., 2000. Atmospheric Pb and Cd input into the Baltic Sea: a new estimate based on measurements. Mar. Chem. 71, 297–307.
Strand, J., Asmund, G., 2003. Tributyltin accumulation and effects in marine molluscs from West Greenland. Environ. Pollut. 123, 31–37.
Strode, E., Jansons, M., Purina, I., Balode, M., Berezina, N.A., 2017. Sediment quality assessment using survival and embryo malformation tests in amphipod crustaceans: the Gulf of Riga, Baltic Sea AS case study. J. Mar. Syst. 172, 93–103.
Strode, E., Barda, I., Suhareva, N., Kolesova, N., Turja, R., Lehtonen, K.K., 2023. Influence of environmental variables on biochemical biomarkers in the amphipod Monoporeia affinis from the Gulf of Riga (Baltic Sea). Water 15, 248.
Sundelin, B., Eriksson, A.K., 1998. Malformations in embryos of the deposit-feeding amphipod Monoporeia affinis in the Baltic Sea. Mar. Ecol. Prog. Ser. 171, 165–180.
Sundelin, B., Ryk, C., Malmberg, G., 2000. Effects on the sexual maturation of the sediment-living amphipod Monoporeia affinis. Environ. Toxicol. 15, 518–526.
Sundelin, B., Rosa, R., Wiklund, A.K.E., 2008a. Reproduction disorders in the benthic amphipod Monoporeia affinis: an effect of low food resources. Aquat. Biol. 2, 179–190.
Sundelin, B., Wiklund, A.K.E., Ford, A.T., 2008b. Biological effects of contaminants : the use of embryo aberrations in amphipod crustaceans for measuring effects of environmental stressors. ICES Tech. Ma.r Environ. Sci. 41, 1–23.
Tairova, Z. and Strand, J., 2022. Biological effect measurments in *Gammarus* spp. and *Corophium volutator* as indicators of toxic effects of hazardous substances in Danish coastal waters. Aarhus University. Technical Report from DCE - Danish Centre for Environment and Energy 237.
Tanner, P.A., James, J., Chan, K., Leong, L.S., 1993. Variations in trace metal and total organic carbon concentrations in marine sediments from Hong Kong. Environ. Technol. (United Kingdom) 14, 501–516.
Tansel, B., Fuentes, C., Sanchez, M., Predoi, K., Acevedo, M., 2011. Persistence profile of polyaromatic hydrocarbons in shallow and deep Gulf waters and sediments: effect of water temperature and sediment-water partitioning characteristics. Mar. Pollut. Bull. 62, 2659–2665.
Tomlinson, D.L., Wilson, J.G., Harris, C.R., Jeffrey, D.W., 1980. Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. Hegoländer Meeresunters 33, 566–575.
Turja, R., Höher, N., Snoeijs, P., Baršiene, J., Butrimavičiene, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen, K.K., 2014a. A multibiomarker approach to the assessment of pollution impacts in two Baltic Sea coastal areas in Sweden using caged mussels (Mytilus trossulus). Sci. Total Environ. 473–474, 398–409.
Turja, R., Lehtonen, K.K., Meierjohann, A., Brozinski, J.M., Vahtera, E., Soirinsuo, A., Sokolov, A., Snoeijs, P., Budzinski, H., Devier, M.H., Peluhet, L., Pääkkönen, J.P., Viitasalo, M., Kronberg, L., 2014b. The mussel caging approach in assessing biological effects of wastewater treatment plant discharges in the Gulf of Finland (Baltic Sea). Mar. Pollut. Bull. 97, 135–149.
Turja, R., Sanni, S., Stankevičiūtė, M., Butrimavičienė, L., Devier, M.H., Budzinski, H., Lehtonen, K.K., 2020. Biomarker responses and accumulation of polycyclic aromatic hydrocarbons in Mytilus trossulus and Gammarus oceanicus during exposure to crude oil. Environ. Sci. Pollut. Res. 27, 15498–15514.
Viglino, L., Pelletier, É., St. Louis, R., 2004. Highly persistent butyltins in northern marine sediments: a long-term threat for the Saguenay Fjord (Canada). Environ. Toxicol. Chem. 23, 2673–2681.
Wiklund, A.K.E., Sundelin, B., 2001. Impaired reproduction in the amphipods Monoporeia affinis and Pontoporeia femorata as a result of moderate hypoxia and increased temperature. Mar. Ecol. Prog. Ser. 222, 131–141.

Publication III

Strode, E., Barda, I., Suhareva, N., Kolesova, N., Turja, R., Lehtonen K. (2023). **Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod *Monoporeia affinis* from the Gulf of Riga (Baltic Sea)**. Water 15(2), 248. https://doi.org/10.3390/w15020248

water

MDPI

Article

Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod *Monoporeia affinis* from the Gulf of Riga (Baltic Sea)

Evita Strode [1,*], Ieva Barda [1], Natalija Suhareva [1], Natalja Kolesova [2], Raisa Turja [3] and Kari K. Lehtonen [3]

[1] Latvian Institute of Aquatic Ecology, Agency of Daugavpils University, Voleru Str. 4, LV-1007 Riga, Latvia
[2] Department of Marine Systems, Tallinn University of Technology, Akadeemia Tee 15a, 12618 Tallinn, Estonia
[3] Marine Research Centre, Finnish Environment Institute SYKE, Latokartanonkaari 11, FI-00790 Helsinki, Finland
* Correspondence: evita.strode@lhei.lv

Abstract: The complexity of the marine environment and the increasing anthropogenic pressure create a necessity to expand existing monitoring approaches. The main goal of this study was to depict the effects of selected, seasonally varying environmental factors on a battery of biomarkers in the benthic amphipod *Monoporeia affinis* from the Gulf of Riga (GoR). Seasonal variability in acetylcholinesterase (AChE), catalase (CAT), glutathione reductase (GR), and glutathione *S*-transferase (GST) activities was investigated at six coastal stations (20–30 m) in August and November in 2020 and 2021. In addition, the biomarkers were measured at seven deep-water stations (>30 m) in November 2021. In general, the results indicated no significant influence of the measured environmental variables on the biomarker activities, except for deep-water stations, where chlorophyll *a* significantly affected enzymatic activity. The current study indicated that *M. affinis* has a higher GST, CAT and GR activity in summer compared to autumn in coastal stations, showing seasonal variability of these biomarkers. However, summarizing the biomarker levels recorded at each station and season, the integrated biomarker response (IBR) index showed the most stressed health status of the *M. affinis* populations in the deep-water stations 135 and 107 and coastal regions in the north-eastern part of the GoR (station 160B). This suggests that the impact on enzymatic responses of benthic organisms could be due to port activities leading to the accumulation of pollutants in muddy sediments regionally. Moreover, for the monitoring of biological effects of contaminants there is a need to establish the background levels of biomarkers, i.e., responses to the different natural environmental factors in the GoR region.

Keywords: amphipods; *Monoporeia affinis*; Gulf of Riga; biochemical biomarkers

check for updates

Citation: Strode, E.; Barda, I.; Suhareva, N.; Kolesova, N.; Turja, R.; Lehtonen, K.K. Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod *Monoporeia affinis* from the Gulf of Riga (Baltic Sea). *Water* **2023**, *15*, 248. https://doi.org/10.3390/w15020248

Academic Editor: Małgorzata Rajfur

Received: 14 December 2022
Revised: 29 December 2022
Accepted: 4 January 2023
Published: 6 January 2023

1. Introduction

Environmental quality control of aquatic ecosystems receives more and more attention due to increasing economic development and rising pollution. Anthropogenic activities are the main factors in increasing the level of contaminants in aquatic environments [1]. As a result, long-term exposure of aquatic organisms to contaminants leads to changes in physiological responses, accumulation of pollutants in tissues, changes in population level, and, finally, changes in species diversity [2]. Applying molecular, biochemical and physiological biomarkers in environmental monitoring programs helps to detect and identify the sub-lethal early-warning effects of pollution on organisms concerning their health, fitness, growth, and reproductive capacity related to ecosystem health in general [3].

Biochemical biomarkers offer information regarding the potential impact of toxic pollutants on the health of organisms and they represent different types of biological responses to different stressors [4]. A large number of chemical contaminants undergo oxidative reactions in cells, leading to the excess production of reactive oxygen species (ROS), which may cause a common phenomenon called oxidative stress [5]. Antioxidant enzyme activity is an important part of the antioxidant defense system (ADS) targeted to prevent oxidative damage to cellular components. In ecotoxicology, the most commonly used enzymes to detect oxidative stress include catalase (CAT), glutathione reductase (GR) as well as the phase II biotransformation enzyme glutathione *S*-transferase (GST) [6,7]. The inhibition of acetylcholinesterase activity (AChE), a key enzyme in the cholinergic nervous system, is a classic biomarker of exposure to neurotoxic compounds, particularly to organophosphates and carbamate pesticides [8], but has been recorded to occur also in connection to exposure to other pollutants such as trace metals, detergents and cyanobacterial toxins [9,10]. These common biomarkers are often used to detect exposure to pollution in aquatic organisms in laboratory and field studies [11].

The selection of suitable organisms for a biomonitoring program is an essential step. Amphipods are considered to be excellent bioindicator organisms as they are widespread over large salinity and habitat ranges [12] and respond to various types of environmental contaminants [6]. They are often an important food source for many fish and invertebrate species, and are thus considered highly relevant organisms for ecotoxicological studies [13]. The amphipod *Monoporeia affinis* (Lindström 1855) is an ecological keystone species of the soft-bottom macrozoobenthic communities in the Baltic Sea and thus a relevant bioindicator organism to be used to monitor and assess anthropogenic impacts [14–17]. Malformed embryos in amphipods are currently recommended as a supplementary indicator of contamination effects on aquatic organisms in the Baltic Sea and are regularly monitored by some countries [13,18,19].

For the correct interpretation of biological responses to chemical contamination, it is necessary to recognize the variability in the applied parameters under different natural environmental conditions such as temperature, dissolved oxygen level, salinity, photoperiod and food availability [20–22]. Variability in some responses is also a natural feature of the annual physiological cycle of the species and can be caused by intrinsic confounding factors such as reproductive status [21,23,24]. Regarding *M. affinis*, the combined effects of pollution in the sediment and oxygen deficiency on ADS responses have been recorded using biochemical biomarkers and reproductive disorders both in laboratory experiments [14] and in field studies [18]. In the Gulf of Riga (GoR), the Baltic Sea subregion of the current study, previous studies regarding the effects of pollution on the growth, reproduction and survival of amphipods have been carried out under standardized laboratory conditions [25–28]. However, biochemical biomarker studies from field-collected samples in the area have been carried out only on the soft-bottom clam *Macoma balthica* (Linnaeus, 1758) and the mussel *Mytilus* spp. [10,20,22,26]. Information on the biological effects of contaminants on amphipods from field studies in the Baltic Sea is relatively scarce and, partly therefore, the application of biological effects methods in monitoring in the region has been underdeveloped until very recently. The research on biochemical biomarkers in *M. affinis* would be the first information in the GoR region and could therefore be used as the basis of regular biomonitoring of contamination effects in the area for future.

The aim of this study was to assess seasonal and spatial variability in selected biochemical biomarkers in *M. affinis* in the GoR as a possible starting point for regular monitoring activities in the area. The above-mentioned ADS response biomarkers CAT, GR and GST were selected to detect possible contaminant-induced oxidative stress while AChE inhibition represents exposure to directly neurotoxic substances or indirect inhibitory effects caused by other types of compounds. The samplings were carried out in 2020 and 2021 in summer and autumn in both years, using a network of sampling sites consisting of shallow coastal and deep-water areas of the GoR. A common set of environmental variables were recorded simultaneously to examine the relationships of these factors with the variability observed in the biomarker levels in the study area.

2. Materials and Methods

2.1. Study Area

The Gulf of Riga is located in the northeastern part of the Baltic Sea and is a semi-enclosed basin with a relatively low salinity of 0.5–7.7 ppt due to its isolation from the Baltic Proper and significant freshwater discharges [29,30]. The GoR surface area is 16.330 km^2 (3.9% of the Baltic Sea area), water volume 424 km^3 (2.1% of the Baltic Sea volume), average depth 26 m, and maximum depth 62 m [31]. Near-bottom water temperature remains low all year round (4–6 °C) [32,33] and dissolved oxygen concentrations range from 2 to 6 mg/L [31,34]. At the depths of 20–30 m, soft bottom substrates or sandy sediments and boulders with well-developed benthic communities prevail with richness up to 13 species, while silty and muddy sediments mostly dominate in the deeper parts (>30 m) of the gulf consisting of only a few amphipod and polychaete species [27,32,35,36].

2.2. Sampling

The *M. affinis* sampling stations were chosen based on the species abundance data available from the Latvian national marine monitoring. The amphipods were collected aboard the R/V "Salme" in August and November in 2020 and 2021 with a Van Veen grab (0.1 m^2) at the depths of 20–43 m (Figure 1) and sieved through a 0.5 mm mesh. The amphipods were immediately frozen in liquid nitrogen, and later placed in a −80 °C freezer for the later biomarker analyses. Salinity, temperature, chlorophyll *a*, and dissolved oxygen concentration, in the near-bottom layer were measured at each site with a multiparameter water quality probe CTD profiler SBE 19 plus SeaCAT (Bellevue, WA, USA).

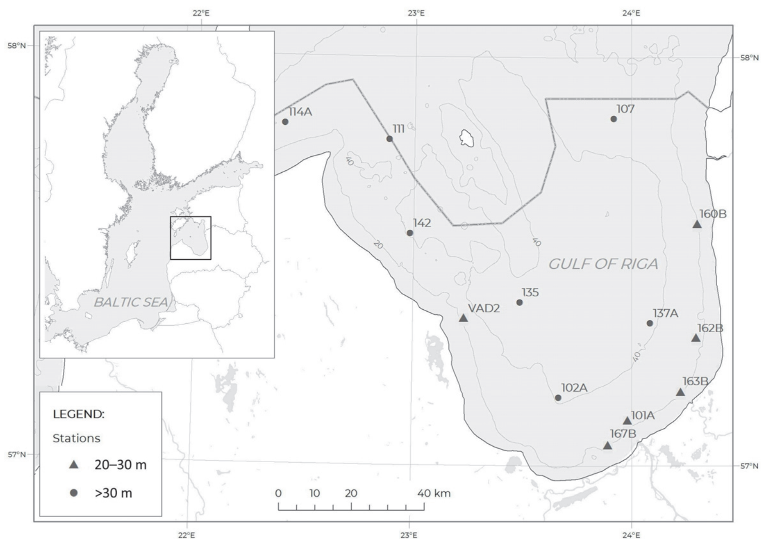

Figure 1. Map of the study area in the Gulf of Riga with markings of coastal (20–30 m) and deep-water (>30 m) stations.

2.3. Sample Preparation and Biomarker Analyses

Whole bodies of five amphipods were pooled for each of the 12 replicate biomarker samples per station. Six replicates were homogenized for 2 × 45 s (Retsch MM400 homogenizer, Haan, Germany) in cold (4 °C) 0.1 M phosphate buffer with 0.15 M KCl (pH 7.4) (1:4 *w*/*v* ratio) to measure the ADS enzyme activities. The remaining six replicates were used to determine AChE with homogenization using cold 0.02 M phosphate buffer with 0.1% Triton X-100 (pH 7.0). During homogenization, the vials were kept on ice. The homogenate was centrifuged at 12,000× *g* at 4 °C for 20 min and the supernatant was used for the assays. Four measurements were carried out per replicate. The enzyme activities were measured with a microplate reader (Spark® Multimode Microplate Reader, TECAN, Grödig, Austria) and analyzed using Magellan software (software version 2.2). The reaction rate was evaluated according to the best linear range of the obtained curve. The specific activities of all the enzymatic biomarkers were calculated against the total protein content of the sample.

The CAT activity was determined according to Claiborne [37]. The supernatant was diluted with the phosphate buffer in a 1:10 (v/v) ratio. The CAT activity was measured recording the decrease of 30 mM H_2O_2 at 240 nm. The measurement of GST activity is based on the conjugation of reduced glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) using a modification of the method based on Habig et al. [38]. The supernatant in a reaction mix (Dulbecco's buffer PBS, 0.1 M GSH and 0.1 M CDNB) were used to measure GST activity at 340 nm. The GR activity level was evaluated as the oxidation rate of NADPH. The supernatant in a reaction mix (0.1 M phosphate buffer + 2 mM EDTA [pH 7.5], 2 mM GSSG, 2 mM NADPH, 3 mM DTNB) was analyzed for GR activity at 412 nm [39]. For the determination of AChE activity according to Ellman et al. [40], the supernatant in 0.02 M phosphate buffer (pH 7.0), 0.1 M acetylcholine iodide (ACTC) and 0.01 M 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) was measured at a 412 nm.

The total amount of protein in the homogenate of each sample was determined with the Bradford assay using bovine serum albumin as the standard [41].

2.4. Statistical Analysis

The biomarker levels were expressed as the mean and standard error (mean ± SE) recorded for amphipods collected from each station by each month, year, and the coastal/deep-water grouping. To ensure homogeneity of data while assessing the seasonal and interannual variability based on the grouped data of the biomarker activity of all coastal stations together, only stations 101A, 167B, VAD2, 163B, 162B were considered in the calculations. The environmental variables recorded were expressed as the mean and standard deviation (mean ± SD). The normality of the biomarker data distribution was checked via the Shapiro–Wilk test. As most of the data were not normally distributed, non-parametric tests were applied. Due to failed data normality, the non-parametric Kruskal–Wallis one-way analysis of variance in combination with the Wilcoxon rank sum test was performed to investigate seasonal and annual variability in medians of biomarker activities across the entire GoR and at the individual sampling stations. Correlations between biomarker levels and physicochemical variables were examined by means of the Spearman's rank correlation and visualized as a correlation matrix using the "corrplot" package of the R software. Data exploration, artworks, and statistical analyses were performed using the R software for Windows, release 4.0.3.

All the measured biomarkers were combined into one general "stress index" known as the Integrated Biomarker Response (IBR) index [42]. The procedure used for the IBR calculation was based on the original paper by Beliaeff and Burgeot [42], modified by Broeg and Lehtonen [43]. The AChE, GST, CAT, and GR data from the two seasons (August and November) in the year 2020 were used for the IBR calculation for the coastal stations while the November data from the year 2021 were used to compare IBR at the coastal and deep-water stations.

3. Results

3.1. Environmental Factors

The measured physicochemical parameters (Table 1) varied moderately between the two seasons (August and November) and the station depth group. The data presented in Table 1 shows that both compared years, 2020 and 2021, differed significantly in terms of water temperature: in August the median in the group of coastal water sites (20–30 m depth) was respectively 8.1 °C and 3.3 °C, while in November it was 10.8 °C and 8.4 °C, respectively. At the coastal stations, higher average values in temperature, chlorophyll *a* and oxygen level were observed in November (9.6 ± 0.4 °C, 2.6 ± 0.2 mg/m^3, and 10.4 ± 0.2 mg/L, respectively) compared to those measured in August (7.5 ± 1.4 °C, 1.3 ± 0.3 mg/m^3 and 5.5 ± 0.5 mg/L, respectively). In addition, in 2021, higher concentrations of dissolved oxygen and chlorophyll *a* in water were recorded in all coastal stations compared to the year 2020. A similar trend was recorded between the depth groups in November, with higher values in temperature, chlorophyll *a* and oxygen level being detected at the coastal stations compared to the deep-water stations (8.1 ± 0.5 °C, 2.2 ± 0.2 mg/m^3, and 9.1 ± 1.2 mg/L, respectively). The average water temperature was slightly lower (by 0.4 °C) in deep-water sites in comparison to coastal sites. At the same time, the median in both groups in (November 2021) of stations (coastal and deep-water) was identical (8.4 °C). However, in November 2021 the higher concentrations of chlorophyll *a* and oxygen content were recorded at coastal stations.

Table 1. Physicochemical parameters measured at the different study stations in the near-bottom layer in August and November 2020 and 2021.

Year	Month	Station	Depth [m]	Temp. [°C]	Absolute Salinity [ppt]	Chlorophyll *a* [mg/m^3]	Oxygen [mg/L]
2020	August	101A	22	7.2	5.8	0.9	4.0
2021			22	3.3	6.1	1.8	5.9
2020	November		22	10.9	6.0	1.6	9.5
2021			23	8.4	6.0	3.7	11.1
2020	August	167B	21	6.4	5.8	0.8	4.7
2021			21	3.2	6.2	1.6	6.4
2020	November		21	10.8	5.9	2.0	9.7
2021			22	8.4	6.0	3.0	11.1
2020	August	163B	22	12.1	5.9	0.9	6.4
2021			22	18.4	5.9	2.2	6.9
2020	November		22	10.8	5.9	2.1	9.7
2021			21	8.5	5.9	3.0	11.1
2020	August	162B	25	8.9	6.0	0.8	4.0
2021			25	3.9	6.1	1.5	6.8
2020	November		25	10.7	5.9	1.9	9.6
2021			24	8.3	5.9	4.0	11.3
2020	August	VAD2	26	5.5	5.9	0.9	4.6
2021			26	3.0	6.2	1.5	8.3
2020	November		26	10.3	5.9	2.3	9.8
2021			25	7.7	6.1	3.0	11.1
2020	August	160B	22	10.1	5.9	0.9	2.9
2020	November		22	10.4	5.7	2.4	9.9
2021	November		22	7.9	5.9	3.0	11.5
2021	November	107	32	8.4	6.0	2.7	11.2
2021	November	111	38	8.8	6.5	2.0	10.7
2021	November	114A	33	8.6	6.8	1.7	10.9
2021	November	142	42	8.2	6.3	2.3	10.7
2021	November	135	45	8.2	6.1	2.6	5.3
2021	November	102A	42	5.3	6.2	1.6	4.0
2021	November	137A	42	9.0	6.0	2.2	10.7

3.2. Seasonal Variability in Biomarkers at the Coastal Stations

In *M. affinis*, the median AChE activity of the pooled data of coastal stations showed no significant variability between August and November ($p > 0.05$) in both year (Figure 2). Although, looking at each station separately, the AChE activity level at station 160B was lower in August than in November in 2020 ($p = 0.020$) and at station 163B in 2021 ($p = 0.01$). Interannual variability could be seen both in August (227.5 ± 7.6 and 191.9 ± 8.3 nmol/min/mg/protein, in 2020 and 2021, respectively, $p = 0.005$) and November (242.1 ± 11.2 and 179.3 ± 7.4 nmol/min/mg/protein, $p < 0.001$).

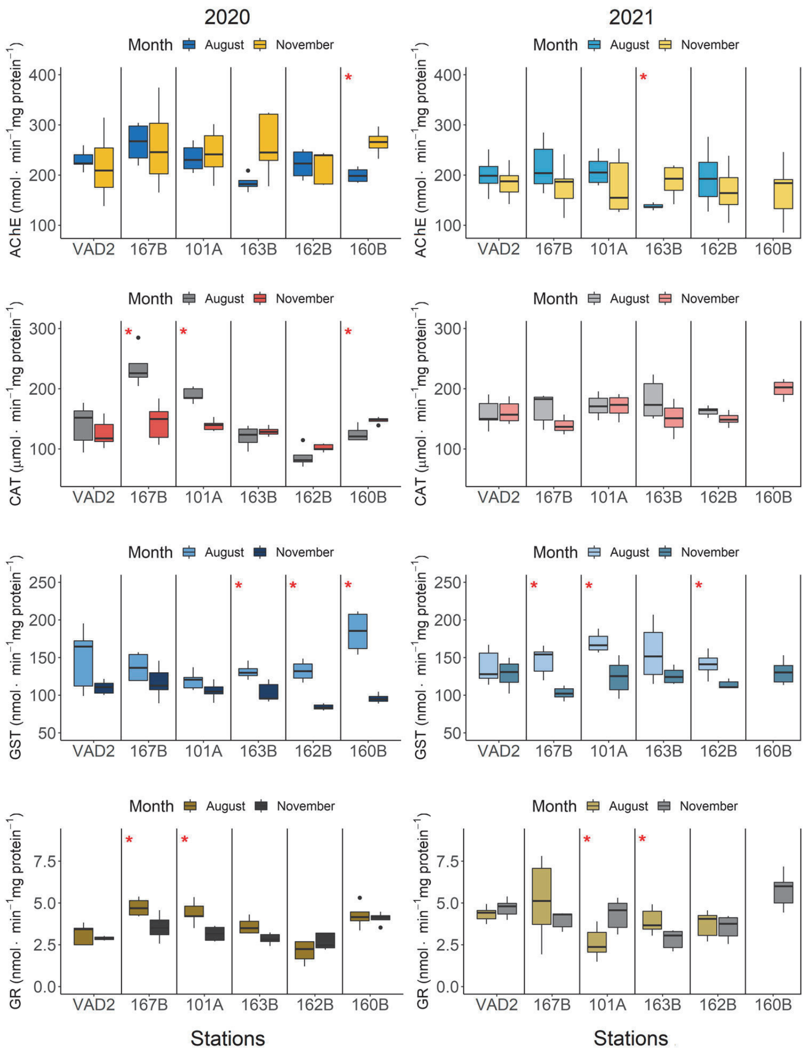

Figure 2. Activity of AChE, CAT, GST, and GR in *M. affinis* at the coastal stations (20–30 m) in the GoR in August and November 2020 and 2021. The red asterisk indicates statistically significant ($p < 0.05$) seasonal differences at the respective stations. Black dots denote outliers calculated according to the Interquartile range (IQR) criterion.

Grouped coastal stations CAT activity did not show any significant variability between August and November in 2020, however it was significant ($p = 0.017$) in 2021 (Figure 2). In 2020, a higher activity was recorded in August at some individual stations, namely 101A ($p = 0.009$), 160B ($p = 0.043$), and 167B ($p = 0.008$). No significant differences between the years could be found in the August samples but in November 2020 the activity was significantly lower ($p < 0.001$) compared to November 2021 (126.9 ± 3.9 and 154.4 ± 3.6 µmol/min/mg/protein, respectively).

In GST, significant interannual (2020 versus 2021) variability has been observed for both August ($p = 0.019$) and November ($p = 0.003$) (Figure 2). Seasonal variability was significant ($p < 0.001$) both for 2020 (133.9 ± 5.1 and 105.1 ± 3.2 nmol/min/mg/protein in August and November, respectively) and 2021 (148.8 ± 3.9 and 123.8 ± 2.4 nmol/min/mg/protein). Seasonal differences in GST activity were significant at most of the coastal stations including 160B ($p = 0.021$), 162B ($p = 0.014$), and 163B ($p = 0.027$) in 2020, and 101A ($p = 0.004$), 162B ($p = 0.010$), 167B ($p = 0.004$) in 2021.

Monoporeia affinis at the individual stations 101A and 167B in 2020 ($p = 0.028$ and 0.037, respectively) and 101A and 163B in 2021 ($p = 0.024$ and 0.016) showed higher GR activity in August (Figure 2). Interannual variability was significant in the November samples where the GR activity across the stations was higher in 2021 (3.9 ± 0.2 nmol/min/mg/protein) compared to 2020 (3.3 ± 0.1 nmol/min/mg/protein) ($p < 0.001$).

3.3. Biochemical Biomarkers at the Deep-Water Stations

Biomarker levels in *M. affinis* at the deep-water stations were measured only in November 2021 (Figure 3). The highest values of AChE activity were observed at station 135 (262.2 ± 24.6 nmol/min/mg/protein), being significantly higher ($p < 0.05$) than at stations 114A, 137A and 111 (115.0 ± 7.8, 140.3 ± 7.1 and 163.9 ± 12.4 nmol/min/mg/protein, respectively).

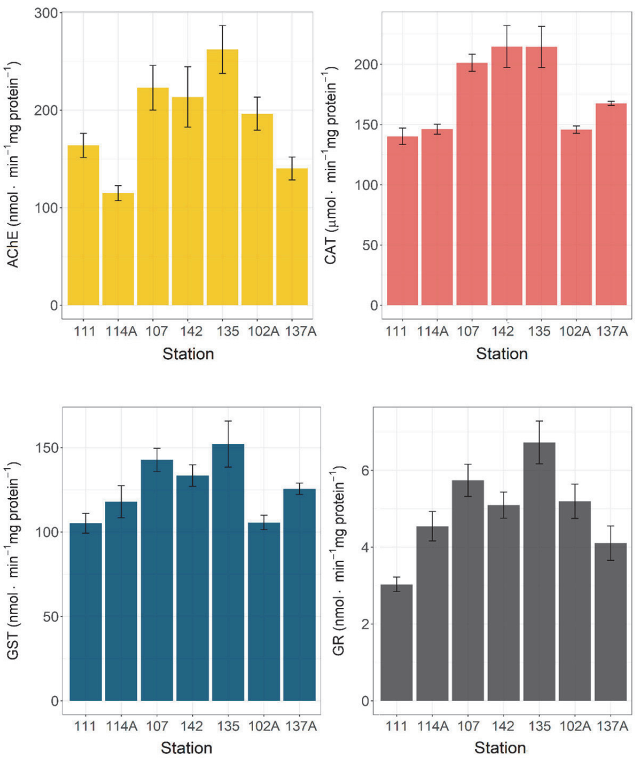

Figure 3. Activity (mean ± SE) of AChE, CAT, GST, and GR in *M. affinis* at the deep-water stations in November 2021.

For CAT, the activity levels were higher at stations 107, 135 and 142 (from 201.3 ± 7.1 to 214.7 ± 17.2 μmol/min/mg/protein) compared to the other four stations ($p < 0.01$).

The GST activity was significantly higher ($p < 0.05$) in samples collected from stations 107 and 135 (142.7 ± 6.9 and 152.1 ± 13.7 nmol/min/mg/protein, respectively), compared to stations 102A and 111 (105.7 ± 4.30 and 105.2 ± 5.9 nmol/min/mg/protein) (Figure 3).

The GR activity was significantly lower (p = 0.011 to 0.031) in samples collected from station 111 (3.0 ± 0.2 nmol/min/mg/protein) compared to the other stations (5.2 ± 0.4, 5.7 ± 0.4, 4.6 ± 0.4, 6.7 ± 0.6, 5.1 ± 0.3 nmol/min/mg/protein for stations 102A, 107, 114A, 135 and 142, respectively), except 137A (4.1 ± 0.5 nmol/min/mg/protein).

Moreover, the activity of CAT and GR among the deep-water stations was significantly higher ($p < 0.001$) than it was among the coastal stations in November 2021.

3.4. Correlation Analysis

Only some of the physicochemical variables showed strong or moderate correlations within a season at the coastal stations (Figure 4). In general, more correlations were found in November compared to August. Near-bottom oxygen concentrations showed a negative correlation with AChE activity of the amphipods both in August and November while being positively correlated with CAT and GR activity in November. Moreover, chlorophyll *a* concentration exhibited a strong negative correlation with AChE and was positive with CAT in November, while no significant relationships between the variables could be observed in August. Regarding temperature, only AChE activity in November showed a significant positive correlation. In the deeper areas of the gulf in November, chlorophyll *a* concentration showed a significant positive correlation with AChE, CAT, and GST (Figure 4).

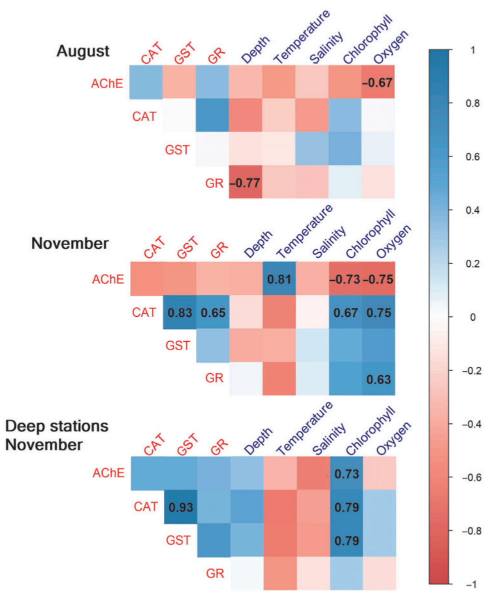

Figure 4. Spearman's rank correlation coefficients between biomarker levels and physicochemical variables were calculated for six coastal stations in August and November, and for seven deep-water stations in November. Correlation coefficients (Spearman's rho) with a $p < 0.05$ are shown and marked based on their sign (+ or −) and strength (continuous color scale).

3.5. Integrated Biomarker Response

The IBR calculations showed variability in the integrated response at the different stations, seasons, and depth ranges (Figure 5). Across the coastal stations, higher index values were detected in August at stations 160B and 167B (Figure 5a). In November, the most impacted amphipod population was observed at the same station 160B while the least impacted was 162B in both seasons. In the comparison of coastal and deep-water station data in November, higher IBR index values were commonly observed at the deep-water stations, especially at 135, 107 and 142, with the notable exception of station 111 (Figure 5b).

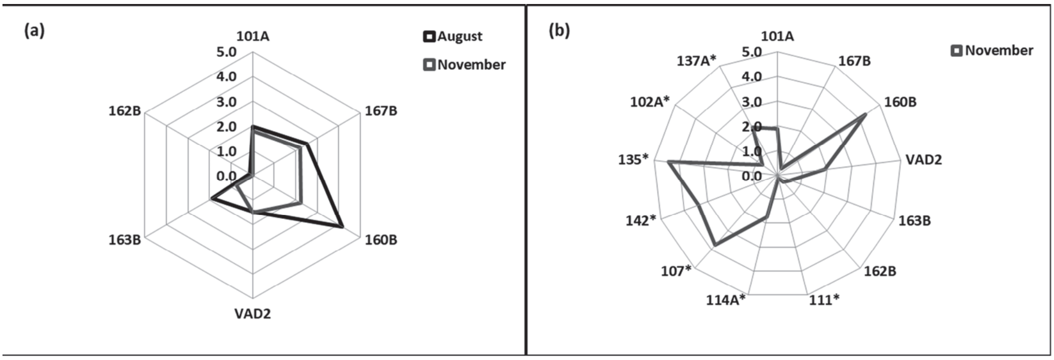

Figure 5. Integrated biomarker response index (IBR) in the amphipod *M. affinis* collected from (**a**) coastal, year 2020 and (**b**) coastal and deep-water stations (marked *), year 2021 from the GoR.

4. Discussion

This study focused on the spatial and temporal differences in selected biomarker responses in a key species in the Gulf of Riga study area and the associations of the responses with some environmental factors. The results obtained provide the first information on biochemical biomarkers in *M. affinis* in this sea area and can therefore be used as the basis of regular biomonitoring of contamination effects in the area.

Seasonal variability has been recognized as an important factor influencing the baseline levels of biomarkers and also the responsiveness of organisms to pollution stress [21,44,45], e.g., in previous studies on *M. balthica* from the GoR [25]. In the present study, seasonal variability in GST and CAT was detected in the amphipods at all the study stations. Of the effects of specific environmental variables measured, the impact of chlorophyll *a* on AChE, CAT, and GST was observed in the samples collected in November in the deeper parts of the GoR. At the same time, a significant negative correlation between chlorophyll *a* in the bottom layer and AChE activity of the amphipods was detected at the coastal stations in November. Normally, a high chlorophyll *a* level in water would indicate good feeding opportunities for filter and deposit feeders and result in an improved physiological condition; however, if associated with toxic phytoplankton blooms such as those regularly occurring in the Baltic Sea in late summer it can cause the inhibition of AChE due to residual cyanobacterial toxins [9,10,14]. Among other abiotic factors, Löf et al. [46] found that both salinity and temperature significantly modified some biomarker responses to contaminants in *M. affinis.* Negative effects of increased temperature during contaminant exposure on *M. affinis*, both on a biochemical and organism level, were recorded also in laboratory conditions [47]. However, in our study, temperature positively and oxygen negatively correlated with AChE activity in November at the coastal stations. A rise in temperature can increase the oxygen consumption of the amphipods, thus increasing energy cost and inhibiting quality of reproductivity [48].

Apart from abiotic variables, biotic factors such as reproductive status, body size and food availability can affect enzymatic responses in amphipods [14,49–51]. Therefore, the physiological status of the organisms should be taken into account in the interpretation of biomarker responses in field studies [52]. *Monoporeia affinis* has a long reproductive cycle; oogenesis starts in late summer and mating takes place in November [53], and the physiological processes during the breeding period lead to the mobilization of energy stores within the organism, potentially increasing their sensitivity to environmental stressors [52]. Significant seasonal differences were also detected in the activity of enzymatic biomarkers between summer and autumn in the clam *M. balthica* from the GoR [20] and the Gulf of Finland [22]. The current study indicated that *M. affinis* has a higher GST, CAT and GR activity in summer compared to autumn. For the amphipod *Hyalella kaingang*, Braghirolli et al. [21] reported a significantly higher CAT activity in summer compared to autumn; however, opposite results were obtained regarding GST activity. Conclusively, the observations above emphasize the importance of being aware of possible different seasonal baselines in biomarkers that can depend on various abiotic and biotic factors [54].

Various biological processes provide information on the types of stressors affecting benthic soft-bottom communities and the reproduction of amphipods possibly related to their observed population decreases in the Baltic Sea [17,55]. Parallel studies in the GoR (unpublished data) indicate that the reproductive success of *M. affinis* is moderately associated with the measured biomarker levels under natural conditions and this information can be utilized when designing early warning monitoring schemes using these parameters. Although the observations on the variability in biomarkers presented in this study are not temporally broad enough to draw conclusions on their long-term dynamics, the significant differences observed in all four biomarker responses between 2020 and 2021 highlight the necessity for longer-term monitoring to reveal the drivers of the observed population declines.

The central part of the GoR (i.e., the deep-water stations) is characterized by a low-diversity benthic community, muddy sediments and relatively high trace metal concentrations [27], where pollutants are relocated and accumulated by currents. The integrated stress response (IBR) in amphipods, assessed by combining the information obtained from all four biomarkers selected for this study, shows a peak in autumn and is more pronounced in the deep-water areas. The elevated biomarker IBR responses were generally observed in *M. affinis* collected from the central region of the GoR (deep-water stations 135, 142) and the north-eastern part of the GoR (stations 160B and 107) as well as at stations situated in the river estuaries. In many cases the biomarker levels were higher at the deep-water stations compared to the coastal area. Elevated trace metal concentrations, particularly of Cd, have been recorded in sediments at the deep-water areas as well as at one sublittoral station (160B) [27]. Obviously, there are also many other contaminants present in the GoR marine environment. Using trace metal levels in sediments as a rough proxy of anthropogenic chemical pollution and their apparent association with the biomarker responses, including the IBR, gives an indication of the usefulness of the biomarker approach when assessing the biological effects of environmental contamination in the area. However, as shown in this study, natural variability has also to be carefully considered when interpreting the results.

5. Conclusions

The current study represents the first comprehensive report on a battery of biochemical biomarkers measured in *M. affinis* collected from the GoR at different seasons and years. The results showed that biological effects methods can be used in environmental quality assessment in this Baltic Sea area. Higher response levels were recorded in populations inhabiting the central (deeper) and northeastern regions of the GoR. Furthermore, fewer differences in biomarker levels were observed in amphipods collected in August compared to November, indicating apparent seasonal variability. The observations highlight the importance of understanding the physiology of a species in its natural habitat, including the periods of greater vulnerability to environmental stressors due to biotic and abiotic factors. Using a specifically designed battery of biomarkers together with measuring a variety of contaminants and key physicochemical variables in monitoring improves our understanding of biochemical responses to environmental stress. This research shows that the use of amphipods for integrated chemical-biological monitoring as well as for the evaluation of sediment quality of the GoR is possible. Finally, the results contribute to the knowledge on the effects of environmental factors on biomarker responses in amphipods and to clarify their dependency on the season that will lead to the development of their natural baseline/threshold values needed in biomonitoring.

Author Contributions: E.S.: conceptualization, data curation, resources, project administration, funding acquisition, formal analysis, validation, visualization, writing—original draft, writing—review and editing; I.B.: conceptualization, resources, validation, visualization, writing—original draft, writing—review and editing; N.S.: data curation, software, visualization, writing—original draft, writing—review and editing. N.K.: validation, writing—review and editing; R.T.: methodology, writing—review and editing; K.K.L.: investigation, methodology, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Regional Development Fund, 1.1.1.2/16/l/001 Post-doctoral project No. 1.1.1.2/VIAA/3/19/465.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments: Our special thanks to the Latvian Institute of Aquatic Ecology laboratory assistants and researchers who participated in the sediment and amphipods sampling, as well as colleagues who made physicochemical analyses.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bashir, I.; Lone, F.A.; Bhat, R.A.; Mir, S.A.; Dar, Z.A.; Dar, S.A. Concerns and Threats of Contamination on Aquatic Ecosystems. In *Bioremediation and Biotechnology*; Springer: Cham, Switzerland, 2020; pp. 1–26. [CrossRef]
2. Hook, S.E.; Gallagher, E.P.; Batley, G.E. The Role of Biomarkers in the Assessment of Aquatic Ecosystem Health. *Integr. Environ. Assess. Manag.* **2014**, *10*, 327–341. [CrossRef]
3. Smit, M.G.D.; Bechmann, R.K.; Hendriks, A.J.; Skadsheim, A.; Larsen, B.K.; Baussant, T.; Bamber, S.; Sanni, S. Relating biomarkers to whole-organism effects using species sensitivity distributions: A pilot study for marine species exposed to oil. *Environ. Toxicol. Chem.* **2009**, *28*, 1104–1109. [CrossRef]
4. Lionetto, M.G.; Caricato, R.; Giordano, M.E. Pollution Biomarkers in Environmental and Human Biomonitoring. *Open Biomark. J.* **2019**, *9*, 1–9. [CrossRef]
5. Stohs, S.J.; Bagchi, D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* **1995**, *18*, 321–336. [CrossRef]
6. Turja, R.; Sanni, S.; Stankevičiūtė, M.; Andreikėnaitė Butrimavičienė, L.; Devier, M.-H.; Budzinski, H.; Lehtonen, K. Biomarker responses and accumulation of polycyclic aromatic hydrocarbons in *Mytilus trossulus* and *Gammarus oceanicus* during exposure to crude oil. *Environ. Sci. Pollut. Res.* **2020**, *27*, 15498–15514. [CrossRef]
7. Vranković, J.; Živić, M.; Radojević, A.; Perić-Mataruga, V.; Todorović, D.; Marković, Z.; Živić, I. Evaluation of oxidative stress biomarkers in the freshwater gammarid *Gammarus dulensis* exposed to trout farm outputs. *Ecotoxicol. Environ. Saf.* **2018**, *163*, 84–95. [CrossRef]
8. Umar, A.M.; Aisami, A. Acetylcholinesterase Enzyme (AChE) as a Biosensor and Biomarker for Pesticides: A Mini Review. *Bull. Environ. Sci. Sustain. Manag.* **2020**, *4*, 7–12. [CrossRef]
9. Kankaanpää, H.; Leiniö, S.; Olin, M.; Sjövall, O.; Meriluoto, J.; Lehtonen, K.K. Accumulation and depuration of cyanobacterial toxin nodularin and biomarker responses in the mussel *Mytilus edulis*. *Chemosphere* **2007**, *68*, 1210–1217. [CrossRef]
10. Lehtonen, K.K.; Kankaanpää, H.; Leiniö, S.; Sipiä, V.O.; Pflugmacher, S.; Sandberg-Kilpi, E. Accumulation of nodularin-like compounds from the cyanobacterium *Nodularia spumigena* and changes in acetylcholinesterase activity in the clam *Macoma balthica* during short-term laboratory exposure. *Aquat. Toxicol.* **2003**, *64*, 461–476. [CrossRef]
11. Lionetto, M.G.; Caricato, R.; Giordano, M.E. Pollution Biomarkers in the Framework of Marine Biodiversity Conservation: State of Art and Perspectives. *Water* **2021**, *13*, 1847. [CrossRef]
12. Whiteley, N.M.; Rastrick, S.P.S.; Lunt, D.H.; Rock, J. Latitudinal variations in the physiology of marine gammarid amphipods. *J. Exp. Mar. Biol. Ecol.* **2011**, *400*, 70–77. [CrossRef]
13. Podlesińska, W.; Dąbrowska, H. Amphipods in estuarine and marine quality assessment—A review. *Oceanologia* **2019**, *61*, 179–196. [CrossRef]
14. Gorokhova, E.; Löf, M.; Reutgard, M.; Lindström, M.; Sundelin, B. Exposure to contaminants exacerbates oxidative stress in amphipod *Monoporeia affinis* subjected to fluctuating hypoxia. *Aquat. Toxicol.* **2013**, *127*, 46–53. [CrossRef]
15. Gorokhova, E.; Löf, M.; Halldórsson, H.P.; Tjärnlund, U.; Lindström, M.; Elfwing, T.; Sundelin, B. Single and combined effects of hypoxia and contaminated sediments on the amphipod *Monoporeia affinis* in laboratory toxicity bioassays based on multiple biomarkers. *Aquat. Toxicol.* **2010**, *99*, 263–274. [CrossRef]
16. Lehtonen, K.K. Seasonal variations in the physiological condition of the benthic amphipods *Monoporeia affinis* and *Pontoporeia femorata* in the Gulf of Riga (Baltic Sea). *Aquat. Ecol.* **2004**, *38*, 441–456. [CrossRef]

17. Sundelin, B.; Eriksson Wiklund, A.-K. Malformations in embryos of the deposit-feeding amphipod *Monoporeia affinis* in the Baltic Sea. *Mar. Ecol. Prog. Ser.* **1998**, *171*, 165–180. [CrossRef]
18. Löf, M.; Sundelin, B.; Bandh, C.; Gorokhova, E. Embryo aberrations in the amphipod *Monoporeia affinis* as indicators of toxic pollutants in sediments: A field evaluation. *Ecol. Indic.* **2016**, *60*, 18–30. [CrossRef]
19. HELCOM. Reproductive Disorders: Malformed Embryos of Amphipods. HELCOM Supplementaryindicator Report. 2018, pp. 1–23, ISSN 2343-2543. Available online: https://helcom.fi/wp-content/uploads/2019/08/Reproductive-disorders-malformed-embryos-of-amphipods-HELCOM-supplementary-indicator-2018.pdf (accessed on 3 January 2023). [CrossRef]
20. Barda, I.; Purina, I.; Rimsa, E.; Balode, M. Seasonal dynamics of biomarkers in infaunal clam *Macoma balthica* from the Gulf of Riga (Baltic Sea). *J. Mar. Syst.* **2014**, *129*, 150–156. [CrossRef]
21. Braghirolli, F.M.; Oliveira, M.R.; Oliveira, G.T. Seasonal variability of metabolic markers and oxidative balance in freshwater amphipod *Hyalella kaingang* (Crustacea, Amphipoda). *Ecotoxicol. Environ. Saf.* **2016**, *130*, 177–184. [CrossRef]
22. Leiniö, S.; Lehtonen, K.K. Seasonal variability in biomarkers in the bivalves *Mytilus edulis* and *Macoma balthica* from the northern Baltic Sea. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2005**, *140*, 408–421. [CrossRef]
23. Benito, D.; Ahvo, A.; Nuutinen, J.; Bilbao, D.; Saenz, J.; Etxebarria, N.; Lekube, X.; Izagirre, U.; Lehtonen, K.K.; Marigómez, I.; et al. Influence of season-depending ecological variables on biomarker baseline levels in mussels (*Mytilus trossulus*) from two Baltic Sea subregions. *Sci. Total Environ.* **2019**, *689*, 1087–1103. [CrossRef]
24. Sheehan, D.; Power, A. Effects of seasonality on xenobiotic and antioxidant defence mechanisms of bivalve molluscs. *Comp. Biochem. Physiol. Part C Pharmacol. Toxicol. Endocrinol.* **1999**, *123*, 193–199. [CrossRef]
25. Berezina, N.A.; Strode, E.; Lehtonen, K.K.; Balode, M.; Golubkov, S.M. Sediment quality assessment using *Gmelinoides fasciatus* and *Monoporeia affinis* (Amphipoda, Gammaridea) in the northeastern Baltic Sea. *Crustaceana* **2013**, *86*, 780–801. [CrossRef]
26. Putna, I.; Strode, E.; Barda, I.; Purina, I.; Rimša, E.; Jansons, M.; Balode, M.; Strake, S. Sediment quality of the ecoregion Engure, Gulf of Riga, assessed by using ecotoxicity tests and biomarker responses. *Proc. Latv. Acad. Sciences. Sect. B. Nat. Exact Appl. Sci.* **2014**, *68*, 20–30. [CrossRef]
27. Strode, E.; Jansons, M.; Purina, I.; Balode, M.; Berezina, N.A. Sediment quality assessment using survival and embryo malformation tests in amphipod crustaceans: The Gulf of Riga, Baltic Sea AS case study. *J. Mar. Syst.* **2017**, *172*, 93–103. [CrossRef]
28. Strode, E.; Balode, M. Toxico-resistance of Baltic amphipod species to heavy metals. *Crustaceana* **2013**, *86*, 1007–1024. [CrossRef]
29. Ojaveer, E. Large-scale processes in the ecosystem of the Gulf of Riga. In *Ecosystem of the Gulf of Riga between 1920 and 1990*; Ojaveer, E., Ed.; Estonian Academy Publishers: Tallinn, Estonia, 1995; pp. 268–277.
30. Skudra, M.; Lips, U. Characteristics and inter-annual changes in temperature, salinity and density distribution in the Gulf of Riga. *Oceanologia* **2017**, *59*, 37–48. [CrossRef]
31. Berzinsh, V. Dynamics of hydrological parameters of the Gulf of Riga. In *Ecosystem of the Gulf of Riga between 1920 and 1990*; Ojaveer, E., Ed.; Estonian Academy: Tallinn, Estonia, 1995; pp. 8–32.
32. Raudsepp, U. Interannual and Seasonal Temperature and Salinity Variations in the Gulf of Riga and Corresponding Saline Water Inflow From the Baltic Proper. *Hydrol. Res.* **2001**, *32*, 135–160. [CrossRef]
33. Yurkovskis, A. Long-term land-based and internal forcing of the nutrient state of the Gulf of Riga (Baltic Sea). *J. Mar. Syst.* **2004**, *50*, 181–197. [CrossRef]
34. Eglīte, E.; Lavrinovičs, A.; Müller-Karulis, B.; Aigars, J.; Poikāne, R. Nutrient turnover at the hypoxic boundary: Flux measurements and model representation for the bottom water environment of the Gulf of Riga, Baltic Sea. *Oceanologia* **2014**, *56*, 711–735. [CrossRef]
35. Kotta, J.; Lauringson, V.; Martin, G.; Simm, M.; Kotta, I.; Herkül, K.; Ojaveer, H. Gulf of Riga and Pärnu Bay. In *Ecology of Baltic Coastal Waters*; Schiewer, U., Ed.; Ecological Studies; Springer: Berlin/Heidelberg, Germany, 2008; pp. 217–243. ISBN 978-3-540-73523-6.
36. Viška, M.; Soomere, T. Simulated and observed reversals of wave-driven alongshore sediment transport at the eastern Baltic Sea coast. *Baltica* **2013**, *26*, 145–156. [CrossRef]
37. Claiborne, A. Catalase Activity. In *CRC Handbook of Methods for Oxygen Radical Research*; CRC Press: Boca Raton, FL, USA, 1985; ISBN 978-1-351-07292-2.
38. Habig, W.H.; Pabst, M.J.; Jakoby, W.B. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **1974**, *249*, 7130–7139. [CrossRef]
39. Smith, I.K.; Vierheller, T.L.; Thorne, C.A. Assay of glutathione reductase in crude tissue homogenates using 5,5′-dithiobis(2-nitrobenzoic acid). *Anal. Biochem.* **1988**, *175*, 408–413. [CrossRef]
40. Ellman, G.L.; Courtney, K.D.; Andres, V.; Featherstone, R.M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, *7*, 88–95. [CrossRef]
41. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]
42. Beliaeff, B.; Burgeot, T. Integrated biomarker response: A useful tool for ecological risk assessment. *Environ. Toxicol. Chem.* **2002**, *21*, 1316–1322. [CrossRef] [PubMed]
43. Broeg, K.; Lehtonen, K.K. Indices for the assessment of environmental pollution of the Baltic Sea coasts: Integrated assessment of a multi-biomarker approach. *Mar. Pollut. Bull.* **2006**, *53*, 508–522. [CrossRef]

44. Jemec, A.; Drobne, D.; Tisler, T.; Sepcic, K. Biochemical biomarkers in environmental studies-lessons learnt from enzymes catalase, glutathione S-transferase and cholinesterase in two crustacean species. *Environ. Sci. Pollut. Res.* **2010**, *17*, 571–581. [CrossRef]
45. Verlecar, X.N.; Jena, K.B.; Chainy, G.B.N. Seasonal variation of oxidative biomarkers in gills and digestive gland of green-lipped mussel *Perna viridis* from Arabian Sea. *Estuar. Coast. Shelf Sci.* **2008**, *76*, 745–752. [CrossRef]
46. Löf, M.; Sundelin, B.; Liewenborg, B.; Bandh, C.; Broeg, K.; Schatz, S.; Gorokhova, E. Biomarker-enhanced assessment of reproductive disorders in *Monoporeia affinis* exposed to contaminated sediment in the Baltic Sea. *Ecol. Indic.* **2016**, *63*, 187–195. [CrossRef]
47. Jacobson, T.; Prevodnik, A.; Sundelin, B. Combined effects of temperature and a pesticide on the Baltic amphipod *Monoporeia affinis*. *Aquat. Biol.* **2008**, *1*, 269–276. [CrossRef]
48. Eriksson Wiklund, A.K.; Sundelin, B. Impaired reproduction in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* as a result of moderate hypoxia and increased temperature. *Mar. Ecol. Prog. Ser.* **2001**, *222*, 131–141. [CrossRef]
49. Chainy, G.B.N.; Paital, B.; Dandapat, J. An Overview of Seasonal Changes in Oxidative Stress and Antioxidant Defence Parameters in Some Invertebrate and Vertebrate Species. *Scientifica* **2016**, *2016*, 6126570. [CrossRef]
50. Glippa, O.; Engström-Öst, J.; Kanerva, M.; Rein, A.; Vuori, K. Oxidative stress and antioxidant defense responses in *Acartia* copepods in relation to environmental factors. *PLoS ONE* **2018**, *13*, e0195981. [CrossRef]
51. Taddei, A.; Räsänen, K.; Burdon, F.J. Size-dependent sensitivity of stream amphipods indicates population-level responses to chemical pollution. *Freshw. Biol.* **2021**, *66*, 765–784. [CrossRef]
52. Sroda, S.; Cossu-Leguille, C. Seasonal variability of antioxidant biomarkers and energy reserves in the freshwater gammarid *Gammarus roeseli*. *Chemosphere* **2011**, *83*, 538–544. [CrossRef]
53. Wiklund, A.-K.E.; Sundelin, B.; Rosa, R. Population decline of amphipod *Monoporeia affinis* in Northern Europe: Consequence of food shortage and competition? *J. Exp. Mar. Biol. Ecol.* **2008**, *367*, 81–90. [CrossRef]
54. Luna Acosta, A.; Bustamante, P.; Godefroy, J.; Fruitier-Arnaudin, I.; Thomas-Guyon, H. Seasonal variation of pollution biomarkers to assess the impact on health status of juvenile Pacific oysters *Crassostrea gigas* exposed in situ. *Environ. Sci. Pollut. Res.* **2010**, *17*, 999–1008. [CrossRef]
55. Rousi, H.; Laine, A.O.; Peltonen, H.; Kangas, P.; Andersin, A.-B.; Rissanen, J.; Sandberg-Kilpi, E.; Bonsdorff, E. Long-term changes in coastal zoobenthos in the northern Baltic Sea: The role of abiotic environmental factors. *ICES J. Mar. Sci.* **2013**, *70*, 440–451. [CrossRef]

Appendix 2

Tabel S1. HELCOM threshold values (2024) for contaminants in sediments included to the HELCOM indicator list.

Compound	**Matrix**	**Threshold value**
Cu (copper)	Sediment	30 mg/kg DW (5%TOC)
Pb (Lead)	Sediment	120 mg/kg DW
Cd	Sediment	2.3 mg/kg DW
TBT	Sediment	1.3 µg/kg DW (5%TOC)
PAHs anthracene	Sediment	24 µg/kg DW
PAHs fluoranthene	Sediment	3500 µg/kg DW, 5% TOC

Curriculum vitae

Personal data

Name:	Natalja Kolesova
Date of birth:	24.08.1983
Place of birth:	Russia
Citizenship:	Estonian

Contact data

E-mail:	natalja.kolesova@taltech.ee

Education

2005–2024	Tallinn University of Technology, PhD
2001–2005	Tallinn University, Marine Biology and Environmental Management, BSc (equalized with MSc)
1991–2001	Kohtla-Järve Tammiku Gymnasium

Language competence

Russian	Native
Estonian	Fluent
English	Upper-Intermediate

Professional employment

2005–present	Tallinn University of Technology, Department of Marine Systems, Specialist in Marine Biology (1.0)

Scientific Work

Publications according to the Estonian Research Information System classification:

1.1

Kolesova, N.; Sildever, S.; Strode, E.; Berezina, N.; Sundelin, B.; Lips, I.; Kuprijanov, I.; Buschmann, F.; Gorokhova, E. (2024). Linking contaminant exposure to embryo aberrations in sediment-dwelling amphipods: a multi-basin field study in the Baltic Sea. Ecological Indicators, 160, #111837. DOI: 10.1016/j.ecolind.2024.111837.

Strode, Evita; Barda, Ieva; Suhareva, Natalija; Kolesova, Natalja; Turja, Raisa; Lehtonen, Kari K. (2023). Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod Monoporeia affinis from the Gulf of Riga (Baltic Sea). Water, 15 (2), #248. DOI: 10.3390/w15020248.

Kuprijanov, I.; Väli, G.; Sharov, A.; Berezina, N.; Liblik, T.; Lips, U.; Kolesova, N.; Maanio, J.; Junttila, V.; Lips, I. (2021). Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland. Marine Pollution Bulletin, 170, #112642. DOI: 10.1016/j.marpolbul.2021.112642.

Sildever, Sirje; Laas, Peeter; Kolesova, Natalja; Lips, Inga; Lips, Urmas; Nagai, Satoshi (2021). Plankton biodiversity and species co-occurrence based on environmental DNA – a multiple marker study. Metabarcoding and Metagenomics, 5, e72371. DOI: 10.3897/mbmg.5.72371.

Kholodkevich, S. V.; Kuznetsova, T. V.; Sharov, A. N.; Kurakin, A. S.; Lips, U.; Kolesova, N.; Lehtonen, K. K. (2017). Applicability of a bioelectronic cardiac monitoring system for the detection of biological effects of pollution in bioindicator species in the Gulf of Finland. Journal of Marine Systems, 171, 151−158. DOI: 10.1016/j.jmarsys.2016.12.005.

Kersen, P.; Kotta, J.; Bučas, M.; Kolesova, N.; Deķere, Z. (2011). Epiphytes and associated fauna on the brown alga Fucus vesiculosus in the Baltic and the North Seas in relation to different abiotic and biotic variables. Marine Ecology, 32, 1, 87−95.

1.2.

Kõuts, T.; Sipelgas, L.; Savinitš, N.; Raudsepp, U. (2007). "Enviromental Monitoring of Water Quality in Coastal Sea Area Using Remote Sensing and Modeling". Environmental Research Engineering and Management, 8−13.

2.5

Rowe,O., Ruiz, M., Wolf, J., Alurralde, G., Blidberg, E., Brockmeyer, B., Munch Christensen, A., Fryer, R., Gorokhova, E., Gustafsson, J., Hüttel, T., Mose Jensen, H., Josefsson, S., Junttila, V., Kaitaranta, J., Klauson, A., Kolesova, N., Kouloumpos, V., Larsen, M. M., Lehtiniemi, M., Murray, C., Naddafi, R., Näslund, J., Pinarbasi, K., Pazdro, K., Poikane, R., Raudkivi, M., Rindorf, A., Sanderson, H., Slobodnik, J., Soerensen, A., Stæhr, P., Strand, J., Tougaard, J., Vähä, E., Ytreberg, E., Zalewska, T. (2023). HELCOM Thematic assessment of hazardous substances, marine litter, underwater noise and non-indigenous species 2016-2021. In: Baltic Marine Environment Protection Commission. (Baltic Sea Environment Proceedings).

Kolesova, N.; Väli, G.; Lips, U. (2021). Conditions that influence Good Environmental Status (GES) in the Baltic Sea. Baltic Marine Environment Protection Commission – Helsinki Commission.

3.1.

Kolesova, N., Siimon, K.-L., Raudsepp, U. (2014). Spatial distribution of macrozoobethos according to environmental conditions in the Lahepere Bay region. Baltic International Symposium (BALTIC), 2014 IEEE/OES, 1−8. DOI: 10.1109/BALTIC.2014.6887884.

Kolesova, N.; Kõuts, M.; Siimon, K.-L.; Raudsepp, U. (2014). Changes in the morphology of Fucus vesiculosus L. and abundance of seaweed associated fauna along the coastal sea of Estonia. Baltic International Symposium (BALTIC), 2014 IEEE/OES, 1−12. DOI: 10.1109/BALTIC.2014.6887832.

Kolesova, Natalja; Raudsepp, Urmas; Alari, Victor (2010). Dominant zoobenthic species in the northwestern coastal sea of Estonia - potential role of abiotic stresses. 4th IEES/OES Baltic Symposium, Riga, Latvia, August 25-27, 2010, 1−8. DOI: 10.1109/BALTIC.2010.5621647.

3.4.

Kõuts, T.; Sipelgas, L.; Savinitš, N.; Raudsepp, U. (2006). Environmental Monitoring Of Water Quality In Coastal Sea Area Using Remote Sensing And Modeling. Proceedings of the US/EU Baltic International Symposium "Integrated Ocean Observation Systems for Managing Global & Regional Ecosystems Using Marine Research, Monitoring & Technologies", Klaipeda, May 23-25, 2006, CD: US/EU Baltic International Symposium "Integrated Ocean Observation Systems for Managing Global & Regional Ecosystems Using Marine Research, Monitoring & Technologies", Klaipeda, May 23-25, 2006. Center of Marine Research, Klaipeda, 1−8.

5.2.

Kuprijanov, Ivan; Kolesova, Natalja; Lipp, Maarja; Lehtonen, Kari K. (2023). Assessing Biological Effects of Contaminants in the Gulf of Finland, Northeastern Baltic Sea, Using Sediment Biotests with Amphipods (Monoporeia affinis) and Biomarker Responses in Clams (Macoma balthica). Proceedings, 2023, EcoBalt 2023: International Conference EcoBalt 2023 "Chemicals & Environment", Tallinn, Estonia, 09–11 October 2023. MDPI, 54. (92). DOI: 10.3390/proceedings2023092054.

Kolesova, Natalja; Kask, Andres; Alari, Victor; Raudsepp, Urmas (2011). The role of abiotic factors on spatial distribution of dominant zoobenthic species in the northwestern coastal sea of Estonia. 8th Baltic Sea Science Congress [BSSC]: 22-26, August 2011, St.Petersburg, Russia : Book of Abstract. St. Petersburg: RSHU, 317.

Kersen, P.; Kotta, J.; Bučas, M.; Kolesova, N.; Deķere, Z. (2009). Macro-epiphytic community patterns of Fucus vesiculosus in the Baltic Sea and the North Sea in relation to different abiotic and biotic variables. Marine Biology in Time and Space: Abstracts from the 44th European Marine Biology Symposium, University of Liverpool, Liverpool. 196 pp.: 44th EMBS, Liverpool, 7-11 september 2009. Ed. Frid, C.L.J., Green, J.A., Paramor, O.A.L., Robinson, L.A. & Watts P.C. Liverpool, 87−87.

Elulookirjeldus

Isikuandmed

Nimi:	Natalja Kolesova
Sünniaeg:	24.08.1983
Sünnikoht:	Venemaa
Kodakondsus:	Eesti

Kontaktandmed

E-post:	natalja.kolesova@taltech.ee

Hariduskäik

2005–2024	Tallinna Tehnikaülikool, PhD
2001–2005	Tallinna Ülikool, Merebioloogia ja keskkonnajuhtimine, BSc (võrdsustatud MSc-ga)
1991–2001	Kohtla-Järve Tammiku Gümnaasium

Keelteoskus

Russian	Emakeel
Estonian	Kõrgtase
English	Kõrgem kesktase

Teenistuskäik

2005–tänaseni	Tallinna Tehnikaülikool, meresüsteemide instituut, merebioloogia spetsialist (1.0)

Teadustegevus

Publikatsioonid Eesti Teadusinfosüsteemi klassifikaatori järgi:

1.1

Kolesova, N.; Sildever, S.; Strode, E.; Berezina, N.; Sundelin, B.; Lips, I.; Kuprijanov, I.; Buschmann, F.; Gorokhova, E. (2024). Linking contaminant exposure to embryo aberrations in sediment-dwelling amphipods: a multi-basin field study in the Baltic Sea. Ecological Indicators, 160, #111837. DOI: 10.1016/j.ecolind.2024.111837.

Kuprijanov, I.; Buhhalko, N.; Eriksson, U.; Sjöberg, V.; Rotander, A.; Kolesova, N.; Lipp, M.; Buschmann, F.; Hashmi, A.; Liblik, T.; Lehtonen, K.K. (2024). A case study on microlitter and chemical contaminants: Assessing biological effects in the southern coast of the Gulf of Finland (Baltic Sea) using the mussel *Mytilus trossulus* as a bioindicator. Marine Environmental Research, 199, #106628. DOI: 10.1016/j.marenvres.2024.106628.

Strode, Evita; Barda, Ieva; Suhareva, Natalija; Kolesova, Natalja; Turja, Raisa; Lehtonen, Kari K. (2023). Influence of Environmental Variables on Biochemical Biomarkers in the Amphipod Monoporeia affinis from the Gulf of Riga (Baltic Sea). Water, 15 (2), #248. DOI: 10.3390/w15020248.

Kuprijanov, I.; Väli, G.; Sharov, A.; Berezina, N.; Liblik, T.; Lips, U.; Kolesova, N.; Maanio, J.; Junttila, V.; Lips, I. (2021). Hazardous substances in the sediments and their pathways from potential sources in the eastern Gulf of Finland. Marine Pollution Bulletin, 170, #112642. DOI: 10.1016/j.marpolbul.2021.112642.

Sildever, Sirje; Laas, Peeter; Kolesova, Natalja; Lips, Inga; Lips, Urmas; Nagai, Satoshi (2021). Plankton biodiversity and species co-occurrence based on environmental DNA – a multiple marker study. Metabarcoding and Metagenomics, 5, e72371. DOI: 10.3897/mbmg.5.72371.

Kholodkevich, S. V.; Kuznetsova, T. V.; Sharov, A. N.; Kurakin, A. S.; Lips, U.; Kolesova, N.; Lehtonen, K. K. (2017). Applicability of a bioelectronic cardiac monitoring system for the detection of biological effects of pollution in bioindicator species in the Gulf of Finland. Journal of Marine Systems, 171, 151−158. DOI: 10.1016/j.jmarsys.2016.12.005.

Kersen, P.; Kotta, J.; Bučas, M.; Kolesova, N.; Deķere, Z. (2011). Epiphytes and associated fauna on the brown alga Fucus vesiculosus in the Baltic and the North Seas in relation to different abiotic and biotic variables. Marine Ecology, 32, 1, 87−95.

1.2.

Kõuts, T.; Sipelgas, L.; Savinitš, N.; Raudsepp, U. (2007). "Enviromental Monitoring of Water Quality in Coastal Sea Area Using Remote Sensing and Modeling". Environmental Research Engineering and Management, 8−13.

2.5

Rowe,O., Ruiz, M., Wolf, J., Alurralde, G., Blidberg, E., Brockmeyer, B., Munch Christensen, A., Fryer, R., Gorokhova, E., Gustafsson, J., Hüttel, T., Mose Jensen, H., Josefsson, S., Junttila, V., Kaitaranta, J., Klauson, A., Kolesova, N., Kouloumpos, V., Larsen, M. M., Lehtiniemi, M., Murray, C., Naddafi, R., Näslund, J., Pinarbasi, K., Pazdro, K., Poikane, R., Raudkivi, M., Rindorf, A., Sanderson, H., Slobodnik, J., Soerensen, A., Stæhr, P., Strand, J., Tougaard, J., Vähä, E., Ytreberg, E., Zalewska, T. (2023). HELCOM Thematic assessment of hazardous substances, marine litter, underwater noise and non-indigenous species 2016-2021. In: Baltic Marine Environment Protection Commission. (Baltic Sea Environment Proceedings).

Kolesova, N.; Väli, G.; Lips, U. (2021). Conditions that influence Good Environmental Status (GES) in the Baltic Sea. Baltic Marine Environment Protection Commission – Helsinki Commission.

3.1.

Kolesova, N., Siimon, K.-L., Raudsepp, U. (2014). Spatial distribution of macrozoobethos according to environmental conditions in the Lahepere Bay region. Baltic International Symposium (BALTIC), 2014 IEEE/OES, 1−8. DOI: 10.1109/BALTIC.2014.6887884.

Kolesova, N.; Kõuts, M.; Siimon, K.-L.; Raudsepp, U. (2014). Changes in the morphology of Fucus vesiculosus L. and abundance of seaweed associated fauna along the coastal sea of Estonia. Baltic International Symposium (BALTIC), 2014 IEEE/OES, 1−12. DOI: 10.1109/BALTIC.2014.6887832.

Kolesova, Natalja; Raudsepp, Urmas; Alari, Victor (2010). Dominant zoobenthic species in the northwestern coastal sea of Estonia - potential role of abiotic stresses. 4th IEES/OES Baltic Symposium, Riga, Latvia, August 25-27, 2010, 1−8. DOI: 10.1109/BALTIC.2010.5621647.

3.4.

Kõuts, T.; Sipelgas, L.; Savinitš, N.; Raudsepp, U. (2006). Environmental Monitoring Of Water Quality In Coastal Sea Area Using Remote Sensing And Modeling. Proceedings of the US/EU Baltic International Symposium “Integrated Ocean Observation Systems for Managing Global & Regional Ecosystems Using Marine Research, Monitoring & Technologies”, Klaipeda, May 23-25, 2006, CD: US/EU Baltic International Symposium “Integrated Ocean Observation Systems for Managing Global & Regional Ecosystems Using Marine Research, Monitoring & Technologies”, Klaipeda, May 23-25, 2006. Center of Marine Research, Klaipeda, 1−8.

5.2.

Kuprijanov, Ivan; Kolesova, Natalja; Lipp, Maarja; Lehtonen, Kari K. (2023). Assessing Biological Effects of Contaminants in the Gulf of Finland, Northeastern Baltic Sea, Using Sediment Biotests with Amphipods (Monoporeia affinis) and Biomarker Responses in Clams (Macoma balthica). Proceedings, 2023, EcoBalt 2023: International Conference EcoBalt 2023 “Chemicals & Environment”, Tallinn, Estonia, 09–11 October 2023. MDPI, 54. (92). DOI: 10.3390/proceedings2023092054.

Kolesova, Natalja; Kask, Andres; Alari, Victor; Raudsepp, Urmas (2011). The role of abiotic factors on spatial distribution of dominant zoobenthic species in the northwestern coastal sea of Estonia. 8th Baltic Sea Science Congress [BSSC]: 22-26, August 2011, St.Petersburg, Russia : Book of Abstract. St. Petersburg: RSHU, 317.

Kersen, P.; Kotta, J.; Bučas, M.; Kolesova, N.; Deķere, Z. (2009). Macro-epiphytic community patterns of Fucus vesiculosus in the Baltic Sea and the North Sea in relation to different abiotic and biotic variables. Marine Biology in Time and Space: Abstracts from the 44th European Marine Biology Symposium, University of Liverpool, Liverpool. 196 pp.: 44th EMBS, Liverpool, 7-11 september 2009. Ed. Frid, C.L.J., Green, J.A., Paramor, O.A.L., Robinson, L.A. & Watts P.C. Liverpool, 87−87.

ISSN 2585-6901 (PDF)
ISBN 978-9916-80-185-7 (PDF)